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LINKS BETWEEN STRESS, SLEEP, AND INFLAMMATION: A TRANSLATIONAL PERSPECTIVE OF RESILIENCE

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LINKS BETWEEN STRESS, SLEEP, AND INFLAMMATION: A TRANSLATIONAL PERSPECTIVE OF RESILIENCE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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A waxing gibbous moon illuminates the tortuous trail as mountaineers climb up Adam's glacier, towards the summit of Mt Adams, in the Cascade Range, Washington. The moon, a reminder that this journey is a passing phase.

— Photo by Joseph K Sabek

Whatever road I take I'm going home…

ABSTRACT

Most individuals will experience one or more extremely traumatic events during their lifetime. For the most part, humans are resilient and have a tremendous capacity to bounce back from hardships; however, for a critical minority, trauma can result in debilitating symptoms, including posttraumatic stress disorder (PTSD). Historically, sleep disturbance and inflammation were viewed as symptoms or consequences of PTSD; however, recently there has been a shift towards conceptualizing sleep disturbance and inflammation as early indications of mental health issues to come. This thesis examined the inextricable link between stress, sleep, and inflammation, and how gaining a better understanding of these interconnected systems could be harnessed to develop enhanced treatments for populations with stress-related symptoms and disorders.

Study I investigated the effect of standardized sleep therapy on posttraumatic stress symptoms, as well as the gene expression pathways that may mediate this effect in sleep disturbed military service members with PTSD (n =39) and controls without PTSD (n = 27). At baseline, participants diagnosed with insomnia and/or obstructive sleep apnea received a combination of 4 to 8 biweekly sessions of cognitive behavioral therapy for insomnia (CBT-I), and automatic positive airway pressure therapy. Results indicated that 22.6% of participants with PTSD had clinically meaningful posttraumatic stress symptom reduction following standardized sleep therapy. Posttraumatic stress symptoms were linked to increased expression of genes associated with immune response systems, which were downregulated with symptom reduction at follow-up.

In order to investigate alternative interventions that may improve sleep quality and potentially provide additional benefits for stress-related disorders, **Study II** was a meta-analysis to determine the effect of mindfulness meditation on sleep quality outcomes in sleep disturbed adults with various mental and physical health conditions. To assess for relative efficacy, mindfulness meditation was compared to evidence-based sleep treatments (like CBT-I and medication) and time/attention-matched interventions to control for placebo effects, which were analyzed separately. The results indicated that mindfulness meditation had a similar effect on sleep quality compared to the evidence-based sleep treatments and was superior to the time/attention matched placebo controls. However, the strength of this evidence was low to moderate, so some doubt remains.

Once mindfulness meditation was established as a potential intervention to improve sleep quality, **Study III** investigated if sleep quality improvements, following a 4-week mindfulness-based integrative medicine program, were associated with reductions in posttraumatic stress, anxiety, depression, and postconcussion symptoms in sleep disturbed military service members with mild traumatic brain injury (n = 93). The secondary aim was to determine if sleep quality improvements were associated with decreases in protein levels of inflammation. Results indicated that sleep quality improvements, following the intervention, were linked to reductions in posttraumatic stress and other neurobehavioral symptoms, but not to inflammation. Moreover, 65.8% of participants with PTSD had clinically meaningful posttraumatic stress symptom reduction at follow-up.

While we found some evidence that posttraumatic stress symptoms were reduced following the mindfulness-based integrative medicine program, this program required almost 30 treatment hours. This may be an excessive treatment duration when mindfulness meditation is used in populations with less severe symptoms. As such, **Study IV** investigated the effect of a brief 5-week (7.5-hour) mindfulness meditation program on perceived stress symptoms in moderately stressed healthcare professionals. Participants were randomized to the mindfulness-based self-care (MBSC) group (n = 43) or the life-as-usual control group (n = 35). Results indicated that the meditation group had larger reductions in perceived stress, and these reductions were maintained two months following the completion of the program.

Taken together, the findings of these four studies led to some important conclusions regarding the link between stress, sleep, and inflammation. While mindfulness research is still in its infancy, these preliminary results suggest that mindfulness meditation is effective in improving sleep in adult populations with various mental and physical health conditions (**Study II**). Less intense mindfulness meditation programs (7.5 hours) may be beneficial to reduce perceived stress, which could potentially prevent the development of more severe mental health conditions (**Study IV**). While 22.6% of individuals with PTSD had reduced posttraumatic stress symptoms following standardized sleep therapy (**Study I**), 65.8% of individuals with PTSD had reduced posttraumatic stress symptoms following the mindfulness-based integrative medicine program (**Study III**). There was some evidence that a relationship exists between sleep quality improvements, decreases in gene expression levels of inflammation, and reductions in posttraumatic stress symptoms; although the direction of causality cannot be determined (**Study I and III**). Clinical implications and recommendations for future research will be discussed.

LIST OF INCLUDED SCIENTIFIC PAPERS

STUDY I **Rusch, H. L.**, Robinson, J., Yun, S., Osier, N. D., Brewin, C. R., & Gill, J. M. (2019). Gene expression differences in PTSD are uniquely related to the intrusion symptom cluster: a transcriptome-wide analysis in military service members. *Brain, Behavior, and Immunity*, *80*, 904-908.

STUDY II **Rusch, H. L.**, Rosario, M., Levison, L. M., Olivera, A., Livingston, W. S., Wu, T., & Gill, J. M. (2018). The effect of mindfulness meditation on sleep quality: a systematic review and meta-analysis of randomized controlled trials. *Annals of the New York Academy of Sciences*, *1445*(1), 5-16.

STUDY III **Rusch, H. L.**, Jiang, A., Guedes, V. A., Haight, T., Lekander, M., Gill, J. M., DeGraba, T.,* & Lasselin, J.,* (2020). Associations between sleep quality, neurobehavioral symptoms, and inflammation among combat-exposed military service members: an observational integrative medicine study. (*contributed equally) *Manuscript*.

STUDY IV Ameli, R., Sinaii, N., Luna, M. J., Panahi, S., Zoosman, M., **Rusch, H. L.**, & Berger, A. (2020). The effects of a brief mindfulness-based program on stress in healthcare professionals in a biomedical research institution: a randomized controlled trial. *JAMA Network Open*, *3*(8), 1-12.

LIST OF RELATED SCIENTIFIC PAPERS

Related scientific papers (denoted with Arabic numerals) are not included in this thesis but are discussed in relation to the current body of work.

PAPER 1 **Rusch, H. L.**, Guardado, P. A., Baxter, T., Mysliwiec, V., & Gill, J. M. (2015). Improved sleep quality is associated with reductions in depression and PTSD arousal symptoms, as well as increases in IGF-1 concentrations. *Journal of Clinical Sleep Medicine*, *11*(6), 615-623.

PAPER 2 Livingston, W. S., **Rusch, H. L.**, Nersesian, P. V., Baxter, T., Mysliwiec, V., & Gill, J. M. (2015). Improved sleep in military personnel is associated with changes in the expression of inflammatory genes. *Frontiers of Psychiatry*, *6*(59), 1-15.

PAPER 3 Guardado, P. A., Olivera, A., **Rusch, H. L.**, Roy, M. J., Martin, C. G., Lejbman, N., Lee, H., & Gill, J. M. (2016). Altered gene expression of the innate immune, neuroendocrine, and NF-κB systems is associated with posttraumatic stress disorder in military personnel. *Journal of Anxiety Disorders*, *38*, 9-20.

PAPER 4 Goyal, M. & **Rusch, H. L.** (2019). Mindfulness-based interventions in the treatment of physical conditions. In Farias, M., Brazier, D., & Lalljee, M. (Eds.), *The Oxford handbook of meditation*. Oxford, U.K.: Oxford University Press.

PAPER 5 Guedes, V., Lai, C., Devoto, C., Edwards, K., Qu, B., **Rusch, H. L.**, Mithani, S., Acott, J. D., Martin, C., Wilde, E. A., Walker, W. C., Diaz-Arrastia, R., Gill, J. M., & Kenney, K. (2020). Exosomal miRNAs and proteins are linked to chronic posttraumatic stress disorder symptoms in service members and veterans. *In Press.*

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropin
ANOVA	Analysis of variance
APAP	Automatic positive airway pressure
BMI	Body mass index
CBT-I	Cognitive behavioral therapy for insomnia
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorder
Dx	Diagnosis
GAD-7	General Anxiety Disorder 7-Item
GLMM	Generalized linear mixed models
HEP	Health Enhancement Program
HPA	Hypothalamic-pituitary-adrenal
ICD	International Classification of Diseases
IFNγ	Interferon gamma
IL	Interleukin
ISI	Insomnia Severity Index
IVT	In vitro
MAAS-S	Mindful Attention Awareness Scale—State
MAAS-T	Mindful Attention Awareness Scale—Trait
MBCT	Mindfulness-based cognitive therapy
MBI-2	Maslach Burnout Inventory 2-Item
MBSC	Mindfulness-based self-care
MBSR	Mindfulness-based stress reduction
MCID	Minimal clinically important difference
MOS-SS	Medical Outcomes Study—Sleep Scale
mRNA	Messenger ribonucleic acid
MSCS-G	Mindful Self-Care Scale—General

mTBI	Mild traumatic brain injury
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
NK	Natural killer
NREM	Non-rapid eye movement
NSI-22	Neurobehavioral Symptom Inventory 22-Item
OSU TBI-ID	Ohio State University, Traumatic Brain Injury Identification Method
PANAS	Positive and Negative Affect Schedule
PCL-M	PTSD Checklist—Military Version
PHQ-9	Patient Health Questionnaire 9-Item
PSQI	Pittsburgh Sleep Quality Index
PSS-10	Perceived Stress Scale 10-Item
PTSD	Posttraumatic stress disorder
REM	Rapid eye movement
RNA	Ribonucleic acid
SAM	Sympathetic–adrenal–medullary
SIMOA	Single Molecule Array HD-1 Analyzer
SWS	Slow-wave sleep
TNF	Tumor necrosis factor
VAS-A	Visual Analog Scale—Anxiety

Let what comes come.
Let what goes go.
Find out what remains.

― Ramana Maharshi

1 INTRODUCTION

Pain and suffering are inextricable experiences of the human condition. Most individuals will experience one or more extremely traumatic events during their lifetime; however, there is significant variability that governs subsequent responses to these events.[1] For the most part, humans are resilient and have a tremendous capacity to bounce back from hardships.[2] However, for a critical minority, trauma can result in debilitating symptoms that impact multiple dimensions of functioning and quality of life.[3] What explains these differences and how can these symptoms best be treated, or better yet be prevented? The answer is only partly understood, but burgeoning evidence points to sleep and inflammation as key players in the pathogenesis and maintenance of stress-related disorders.

1.1 STRESS

In 1950, Hans Selye first defined stress from a biological perspective as a nonspecific response of the body to any demand made upon it (e.g., exercise, extreme temperatures).[4] Not all scientists agreed with Selye's definition; they argued that if the stress response was nonspecific then everyone should react the same way to the same stressor.[5] In the 1960s, cognitive processes were integrated into the biological model of stress to resolve this discrepancy. It was determined that individual differences in perception account for these diverse responses to a stressor—stress is not what happens to you, but how you respond to it.[6] Despite this reconciliation, there are a number of universal conditions that elicit a stress response in almost everyone: novelty, unpredictability, and loss of control.[7] The terms eustress (i.e., good stress) and distress (i.e., toxic stress) were later introduced to distinguish the stress response triggered by a positive event from a negative event (Figure 1).[8,9]

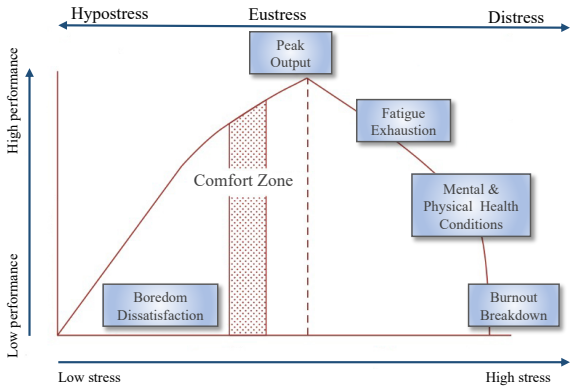


Figure 1 | The human function curve. Eustress improves performance, creativity, and growth. Meanwhile, distress causes fatigue and exhaustion followed by the onset of mental and physical health conditions, which impede performance. Adapted from "*The human function curve: a paradigm for our times*", by P.G. Nixon, 1982, Activitas Nervosa Superior, p. 133.

1.1.1 Effects of acute stress

When an event is perceived as a stressor, the brain and body recruit a range of complex systems to maintain homeostasis.[10] This adaptive stress response is called allostasis—achieving stability through change.[11] The first phase of the stress response is initiated by the central nervous system, whereby physical stressors (e.g., viral infections) are mainly processed by the brainstem and limbic regions, and psychosocial stressors (e.g., public speaking) are largely processed by the executive, affective, and salience networks, including the prefrontal cortex, amygdala, and hippocampus.[12-14] Next, the autonomic nervous system is activated, namely, the sympathetic–adrenal–medullary (SAM) axis, which culminates in the release of epinephrine and norepinephrine (collectively catecholamines) into the bloodstream by the adrenal medulla.[15] Then the hypothalamic-pituitary-adrenal (HPA) axis is activated, in which corticotropin-releasing hormone (CRH) is secreted from the paraventricular nucleus in the hypothalamus.[16] This elicits the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, prompting the synthesis and release of glucocorticoids (cortisol in humans) into the bloodstream by the adrenal cortex.[16] The increase of circulating catecholamines and glucocorticoids coordinates widespread changes in vast systems (e.g., immune, cardiovascular, metabolic) to protect and prepare the body for a fight-or-flight response.[17] Once the perceived stressor has passed, feedback loops are triggered in various systems (e.g., from the adrenal gland to the hypothalamus) to terminate the stress response and restore homeostasis.[18]

1.1.2 Effects of chronic stress

When the stress response becomes prolonged or excessive, it can put wear and tear on the brain and body (i.e., allostatic overload) and cause alterations in physical, cognitive, emotional, and behavioral functioning (Figure 2).[19] Within the immune system, a persistent influx in glucocorticoids may result in glucocorticoid receptor resistance, which in turn, interferes with the downregulation of inflammation.[20] The combination of HPA axis dysregulation and increased inflammation might converge with other brain and body systems to contribute to psychiatric and immune-mediated disorders (e.g., autoimmune, cardiovascular, metabolic).[21,22] The brain not only can affect the immune system, but the immune system can affect the brain; this bi-directional communication can result in increased neuroinflammation under chronic stress.[23] Increased neuroinflammation while initially adaptive, if left unchecked can result in aberrant functional and structural changes in brain regions key to memory and emotional regulation.[24,25] For example, acute stress often enhances memory—a possible survival mechanism to help remember a dangerous

situation—however, chronic stress often impairs memory function.[26,27] Prolonged stress can increase the risk for burnout—a syndrome defined by symptoms of emotional exhaustion and depersonalization.[28,29] If excessive, early life stress may increase the risk for impaired social skills, aggression, addiction (e.g., food, nicotine, alcohol, drugs), and posttraumatic stress disorder (PTSD) in adulthood.[30-33]

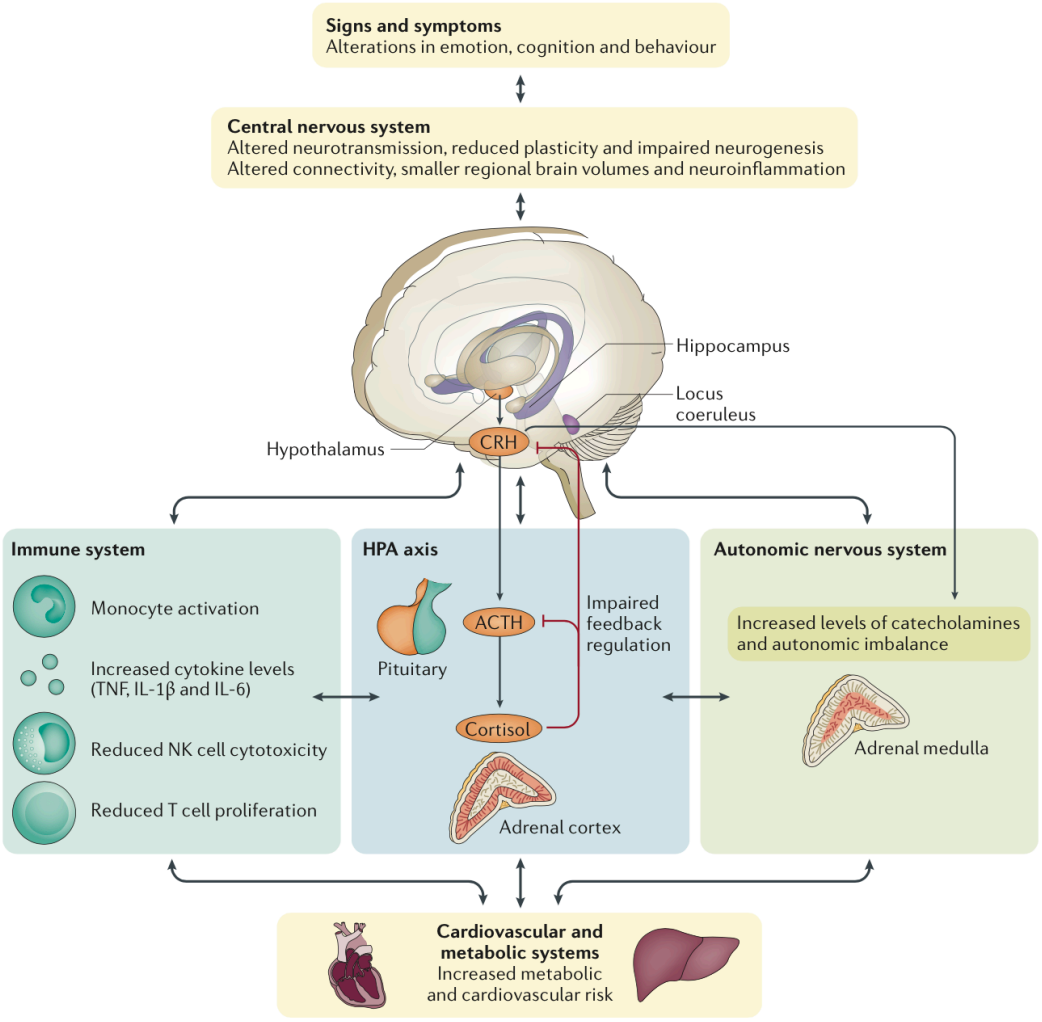


Figure 2 | Biological systems involved in the pathophysiology of chronic stress. In the brain, chronic stress may result in functional changes in brain circuits (for example, in executive, affective, and salience networks), structural changes in brain volumes (for example, in the hippocampus), and increased neuroinflammation. Beyond the brain, chronic stress impairs feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Within the immune system, there is evidence of increased levels of circulating cytokines and low-grade activation of innate immune cells, including monocytes. The combination of HPA axis dysregulation and increased inflammation might converge with other brain and body systems to contribute to psychiatric and immune-mediated disorders. The sequence of events leading to changes in these interconnected systems is not known; however, alterations in the stress response system can, directly and indirectly, affect the brain and other body systems, which suggests a bidirectional link. ACTH, adrenocorticotropin; CRH, corticotropin-releasing hormone; IL, interleukin; NK, natural killer; TNF, tumor necrosis factor. Adapted from "*Major depressive disorder*", by C. Otte, et al., 2016, Nature Reviews Disease Primers, 2, p. 8. © 2016 by Macmillan Publishers Limited, part of Springer Nature. Adapted with permission.

1.2 POSTTRAUMATIC STRESS DISORDER

Posttraumatic stress reactions have roots stretching back centuries and were widely known to previous generations as combat fatigue, shell shock, soldier's heart, or war neurosis.[34] However, it was not until 1980 that PTSD became a household name and was officially recognized in the third edition of the Diagnostic and Statistical Manual of Mental Disorder (DSM-III).[35] Since then our understanding of PTSD has grown significantly; nonetheless, the field of traumatic stress is still riddled with controversy over the actual diagnostic features of PTSD, its causation, and what constitutes evidence-based treatment.[36-38]

1.2.1 Diagnostic features

Currently, there are two recognized definitions of PTSD—the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), published in 2013 by the American Psychiatric Association, and the International Classification of Diseases, 11th Revision (ICD-11), released in 2018 by the World Health Organization (Table 1). Both PTSD definitions require that the individual witnesses or experiences firsthand an extremely traumatic event (e.g., actual/threatened death, severe injury, or sexual violence). If that criterion is met, the DSM-5 requires the manifestation of at least one of five intrusion symptoms, one of two avoidance symptoms, two of seven alterations in cognitions and mood, and two of six alterations in arousal and reactivity.[39] Instead, the ICD-11 requires the manifestation of at least one of two intrusion symptoms, one of two avoidance symptoms, and one of two alterations in arousal and reactivity.[40] These symptoms must persist for at least one month, cause clinically significant distress or impairment in social, occupational, or other key areas of functioning, and not be attributed to the effects of a substance (e.g., alcohol, medication) or another medical condition (e.g., hypothyroidism, seizure).[39]

1.2.2 Diagnostic discrepancies

Historically, the DSM and ICD have been concordant with their PTSD definitions. However, in the latest revisions, the DSM-5 expanded its scope to include three new symptoms, while the ICD-11, in a strategic effort to improve diagnostic accuracy, narrowed its focus to the core fear-based symptoms of PTSD.[41,42] The effect of these modifications is clinically significant, as several studies report that more individuals are diagnosed with PTSD using the DSM-5 criteria compared with the ICD-11 criteria.[43,44] Moreover, both definitions identify different individuals as having PTSD.[45] Another concern surrounds the inherent virtues of the two diagnostic definitions. On one side, the DSM-5 criteria apply to a larger population of trauma-exposed individuals with more diverse symptoms; however,

the inclusion of broader symptoms may increase the rate of misdiagnosing other mental health conditions as PTSD.[45-50] This has serious implications for making treatment recommendations and for the discovery of meaningful biomarkers.[51]

Table 1 | Comparison of the DSM-IV, DSM-5, and ICD-11 symptom criteria for PTSD.

Clusters	Symptoms	DSM-IV	DSM-5	ICD-11
INTRUSION	1. Repeated, disturbing, and unwanted memories of the stressful experience	√	√	
	2. Repeated, disturbing dreams of the stressful experience	√	√	√
	3. Suddenly feeling or acting as if the stressful experience were happening again	√	√	√
	4. Feeling very upset when something reminded you of the stressful experience	√	√	
	5. Having strong physical reactions when something reminded you of the stressful experience	√	√	
AVOIDANCE	1. Avoiding memories, thoughts, or feelings related to the stressful experience	√	√	√
	2. Avoiding external reminders of the stressful experience	√	√	√
ALTERATIONS IN MOOD AND COGNITION	1. Trouble remembering important parts of the stressful experience	√	√	
	2. Having strong negative beliefs about yourself, other people, or the world		√	
	3. Blaming yourself or someone else for the stressful experience or what happened after it		√	
	4. Having strong negative feelings such as fear, horror, anger, guilt, or shame		√	
	5. Loss of interest in activities you used to enjoy	√	√	
	6. Feeling distant or cut-off from other people	√	√	
	7. Trouble experiencing positive feelings	√	√	
ALTERATIONS IN AROUSAL AND REACTIVITY	1. Irritable behavior, angry outbursts, or acting aggressively	√	√	
	2. Taking too many risks or doing things that could cause you harm	√	√	
	3. Being "super alert" or watchful or on-guard	√	√	√
	4. Feeling jumpy or easily startled	√	√	√
	5. Having difficulty concentrating	√	√	
	6. Trouble falling or staying asleep	√	√	

Symptoms are reproduced from the PTSD Checklist for DSM-5, a 20-item self-report measure that assesses the presence and severity of PTSD symptoms. The 'Avoidance' and 'Alterations in Mood and Cognition' symptom clusters are combined into one Avoidance/Numbing symptom cluster for the DSM-IV. DSM, Diagnostic and Statistical Manual of Mental Disorders; ICD, International Classification of Diseases; PTSD, posttraumatic stress disorder

1.2.3 Prevalence

The 2014 World Mental Health Survey reported 12-month PTSD prevalence rates at 1.1% across all respondents (N = 51,295), and at 4.0% in respondents with trauma exposure (N = 47,466).[52,53] PTSD prevalence rates are significantly higher in high-income countries (compared to low), as well as in respondents exposed to sexual assault, physical assault,

and organized violence (compared to other trauma types).[52,54] The 2014 National Health Study for a New Generation of U.S. Veterans (N = 20,563) reported PTSD prevalence rates at 13.5% across all military populations, at 15.7% in deployed veterans, and at 10.9% in nondeployed veterans.[55] Prevalence estimates were higher in deployed men than deployed women (16.0% vs. 12.5%) but lower in nondeployed men than nondeployed women (10.5% vs. 12.3%).[55] Other studies found sex differences across trauma type (Figure 3). For example, in the United States, the risk of developing PTSD is higher in men than women after rape (65.0% vs. 45.9%) but lower in men than women after a physical attack (1.8% vs. 21.3%).[56] These disparities may reflect sex and societal role differences that play into the development, expression, and progression of posttraumatic stress symptoms.[57]

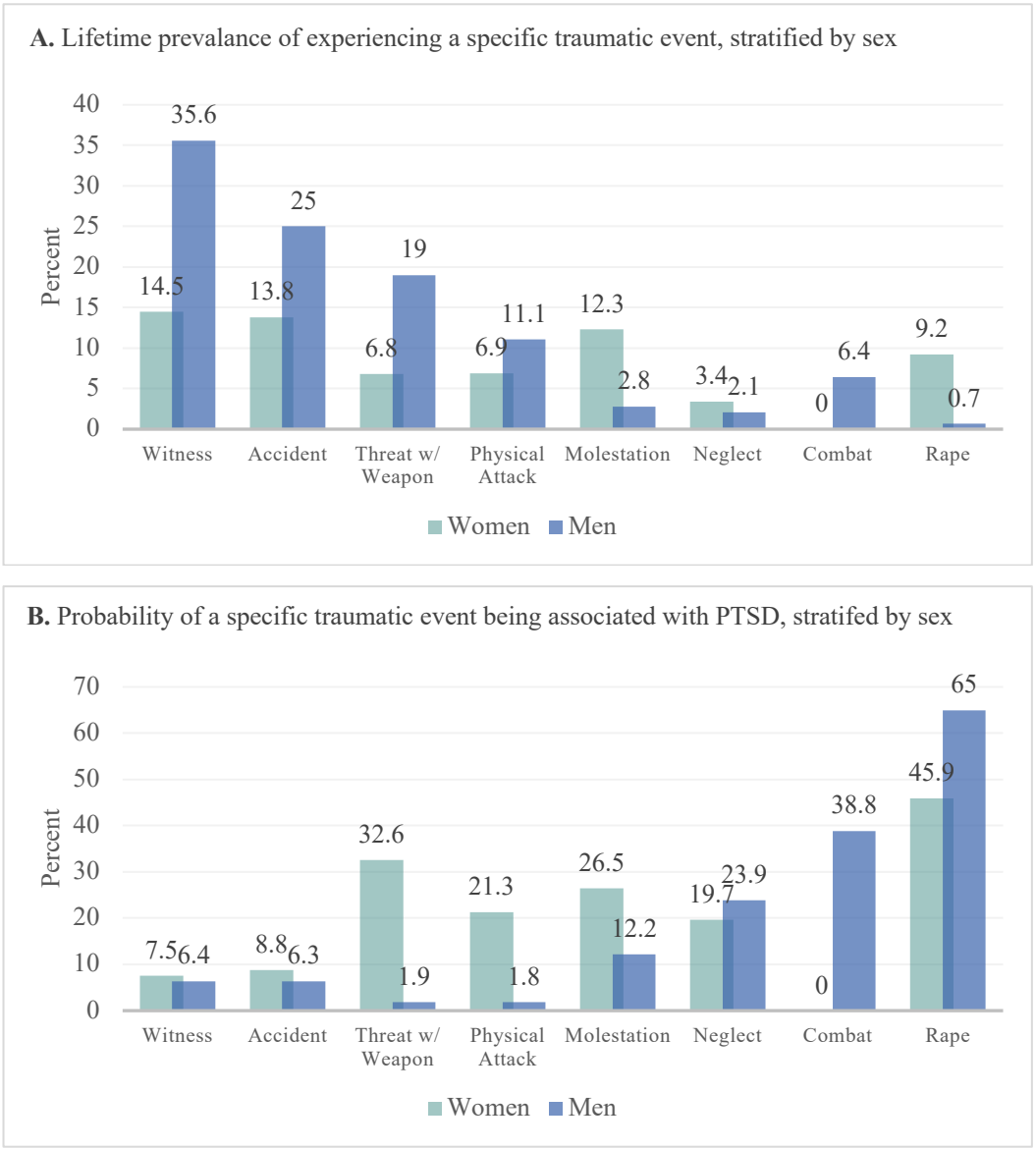


Figure 3 | Sex differences in trauma exposure and posttraumatic stress disorder (PTSD) in the United States. (A) Lifetime prevalence of trauma exposure stratified by sex. (B) The proportion of women and men with a specific trauma exposure who met criteria for PTSD.[56]

1.2.4 Comorbidities

Individuals who meet the criteria for one health condition more often meet the criteria for other health conditions—a phenomenon known as comorbidity. The National Comorbidity Survey (*N* = 5,877) found that over 88% of men and 79% of women with PTSD were diagnosed with at least one other mental health condition.[56] The most common concurrent mental health diagnoses were major depression (37.2%), panic disorder (33.3%), social phobia (19.9%), and substance dependence (10.2%).[58] Compared to individuals without PTSD, people with PTSD were at increased risk for autoimmune, cardiovascular, digestive, metabolic, musculoskeletal, and neurodegenerative disorders, as well as life-threatening infections.[59-62] The elevated occurrence of comorbid conditions seen in PTSD is likely mediated by a combination of genetic and environmental factors; however, the precise contribution of these mediators is still widely unknown. There is also a substantial symptom overlap between PTSD and comorbid conditions—especially mild traumatic brain injury (mTBI) in military populations—so the high rate of comorbidity could partly result from an epiphenomenon of the diagnostic criteria.[63]

1.3 Sleep

Sleep is an essential human behavior that is characterized by a reversible state of diminished receptiveness and loss of consciousness, which is typically initiated in a recumbent position with eyes closed.[64] Recent advances in sleep research indicate that sleep may play a critical role in emotional regulation, cognitive function (including memory processing and consolidation), immune regulation, and brain waste removal.[65,66] Adequate and quality sleep supports the functioning of almost every type of tissue and system in the body. Meta-analyses (*N*s > 2,000,000) found that insufficient sleep (less than 7 hours) and prolonged sleep (more than 9 hours) were associated with increased risk for all-cause morbidity and cardiovascular events.[67,68] Sleep is regulated by a two-process system comprised of sleep-wake homeostasis and circadian rhythms.[69] Sleep-wake homeostasis means that sleep intensity and duration increases after a prolonged period without sleep.[70] In other words, the greater the sleep deficit, the greater the need to fall asleep and stay asleep longer. Circadian rhythms, on the other hand, modulate the timing of sleep. The central circadian clock is located in the suprachiasmatic nucleus, a small region of the brain in the hypothalamus. This system is responsible for imposing and synchronizing a close to 24-hour rhythm on multiple bodily systems, including the proclivity to be awake or asleep at a particular time.[70]

1.3.1 Stages of sleep

Sleep is divided into distinct stages marked by a discrete pattern of brain activity that can be measured by electroencephalography during polysomnography (i.e., sleep study). Rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep are the two main stages of sleep. In humans, NREM sleep is further divided into three stages, N1, N2, and N3 (formerly stages 3 and 4), which correspond to a range of sleep depths.[71] Stage N1 marks the transition between wakefulness and sleep, which is typically followed by stage N2—collectively, these are lighter stages of sleep.[72] Stage N3, also referred to as slow-wave sleep (SWS) due to the preponderance of high-amplitude, low-frequency waves, is the deepest of all sleep stages.[72] The fourth stage is REM sleep, brain activity during REM sleep resembles the waking brain and most dreaming occurs during this sleep stage.[72] An average night of sleep contains four to six NREM-to-REM sleep cycles, in which each cycle lasts about 80 to 110 minutes.[72] Stage N3 sleep (i.e., SWS) predominates during the first half of the night, while REM sleep increases as the night progresses (Figure 4A).[72] The different stages of sleep are defined by specific brain oscillations measured by polysomnography (Figure 4B). For example, SWS sleep is characterized by slow oscillations (0.5-4 hertz), slow spindles (9-12 hertz), and fast spindles (12-15 hertz).[73]

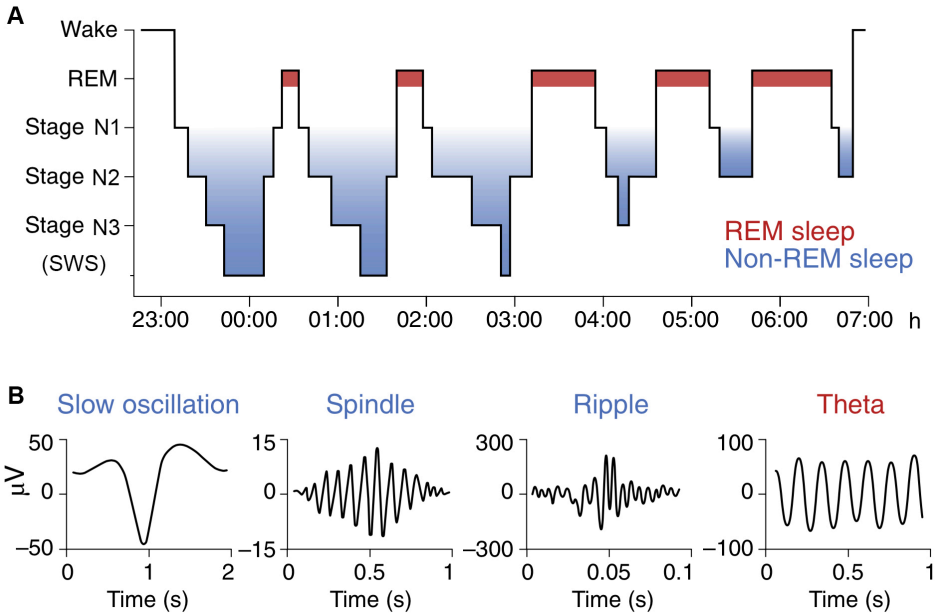


Figure 4 | Sleep architecture and sleep oscillations. (A) Human nocturnal sleep profile. (B) Theta oscillations are prominent during rapid eye movement (REM) sleep. Neocortical slow oscillations, thalamocortical spindles, and hippocampal ripples are signatures of non-rapid eye movement (NREM) sleep. Slow oscillations and spindles are hallmark features of slow-wave sleep (SWS). Adapted from "*Mechanisms of systems memory consolidation during sleep*", by J. G. Klinzing, et al., 2019, Nature Neuroscience, 22, p. 1603. © 2019 by Springer Nature America, Inc. Adapted with permission.

1.3.2 Measurements of sleep

Sleep quality is determined by a collection of sleep parameters, most commonly, total sleep time, sleep onset latency (the time it takes to fall asleep once in bed), wake after sleep onset (the time spent awake after initially falling asleep), and sleep efficiency (the percentage of time spent asleep while in bed).[74] Sleep quality can be measured objectively (by instrument) or subjectively (by self-report). The most common objective measures used in clinical and research settings are the polysomnography and actigraphy. Traditionally, the laboratory-based polysomnography is considered the gold standard measure of sleep quality; however, it is impractical for long-term use and home utilization (e.g., resource-demanding, difficult to use).[75] During polysomnography, electrodes and sensors are placed on the body to monitor brain activity, muscle activity, eye movement, heart rhythm, respiratory rate, blood pressure, and blood oxygen levels. Actigraphy, on the other hand, is a watch-like device that measures gross motor activity from which estimates of sleep-wake patterns are made. Actigraphy has similar validity to polysomnography in assessing sleep parameters in healthy populations but tends to overestimate sleep quality in sleep disturbed populations.[76] This is partly due to mischaracterizing states of laying still while awake as states of sleep.[76] There are several questionnaires that measure subjective sleep quality retrospectively (e.g., over the past month) or diaries that measure subjective sleep quality prospectively (e.g., every night for the next week). The correlation between objective and subjective measures of sleep quality is low, which suggests that the two methods measure different dimensions of sleep.[77] Still, other theories have suggested that individuals with insomnia experience 'sleep state [mis]perception'—a trend to underestimate total sleep time and overestimate sleep onset latency.[78,79] Nonetheless, subjective measures of sleep quality (compared to objective measures) seem to be better predictors of health outcomes in at least some clinical populations, including PTSD.[80,81]

1.3.3 Link between sleep and posttraumatic stress

Sleep disturbances, including difficulties initiating and maintaining sleep, are reported in over 70% of individuals with PTSD.[82] Burgeoning evidence suggests that sleep disturbance is not merely a symptom of PTSD but instead plays a role in the development and maintenance of posttraumatic stress symptoms.[83] Multiple longitudinal studies have indicated that pre-trauma or peri-trauma sleep disturbances contribute to the prediction of new-onset PTSD,[84-89] with meta-analytic findings reporting that sleep disturbance yields a three-fold increased risk for anxiety.[90] For example, in a longitudinal study of National Guard Soldiers (N = 522), predeployment daytime and nighttime sleep complaints

contributed to the prediction of new-onset PTSD at two years postdeployment.[91] While sleep disturbance is reported to predict future PTSD, baseline posttraumatic stress symptoms did not predict subsequent sleep disturbance or even subsequent PTSD.[92,93] Evidence of polysomnography-measured sleep irregularities in PTSD were also found in two meta-analyses.[94,95] The first study (N = 722) reported increased stage N1 sleep (i.e., light sleep) and decreased SWS (i.e., deep sleep) in participants with PTSD compared to controls.[94] The second study (N > 2000) also reported decreased SWS, which was associated with PTSD symptom severity; however, no difference in stage N1 sleep was found.[95] Accumulating evidence also supports the inhibition and/or disruption of REM sleep in the acute phase of PTSD, which hints at a possible pathogenic marker of symptom progression.[96-100] Taken together, these findings demonstrate the interplay between sleep quality and PTSD; however, the extent to which posttraumatic stress symptoms can be mitigated by targeting sleep disturbance directly is less clear.

1.4 INFLAMMATION

The immune system is the body's defense system against pathogens. Its main purpose is to identify and remediate internal and external threats like bacteria, fungi, parasites, viruses, and injury. Cells of the immune system circulate throughout the body and are roughly grouped into two systems: the innate immune system and the adaptive immune system. The innate immune system responds rapidly to threats upon the first encounter. Meanwhile, the adaptive immune system takes several days to respond but does so in a tailor-specific way that is sustained by memory cells that prime for threat reencounter.[70] When the innate immune system detects a threat, it initiates a cascade of inflammatory responses to contain the spread of infection and stimulate healing to damaged tissue.[101] The inflammatory mediators involved in these responses are cytokines, chemokines, prostaglandins, and vasoactive amines, which incite an array of processes aimed at restoring homeostasis.[101] Locally, these processes result in clinical signs of inflammation (i.e., redness, swelling, warmth, pain, and impaired function), rapid release of antimicrobials, and phagocytosis.[70] Systemically, these processes result in symptoms like fever and sickness behavior, which is characterized by anorexia, adipsia, anxiety, anhedonia, fatigue, pain sensitivity, sleep alterations, and social withdrawal.[70,102] Under healthy conditions, acute inflammation is a protective mechanism for the body. On the other hand, when chronically sustained, inflammation can cause serious damage to the organism's own tissues and organs, including the brain (Figure 5).[103]

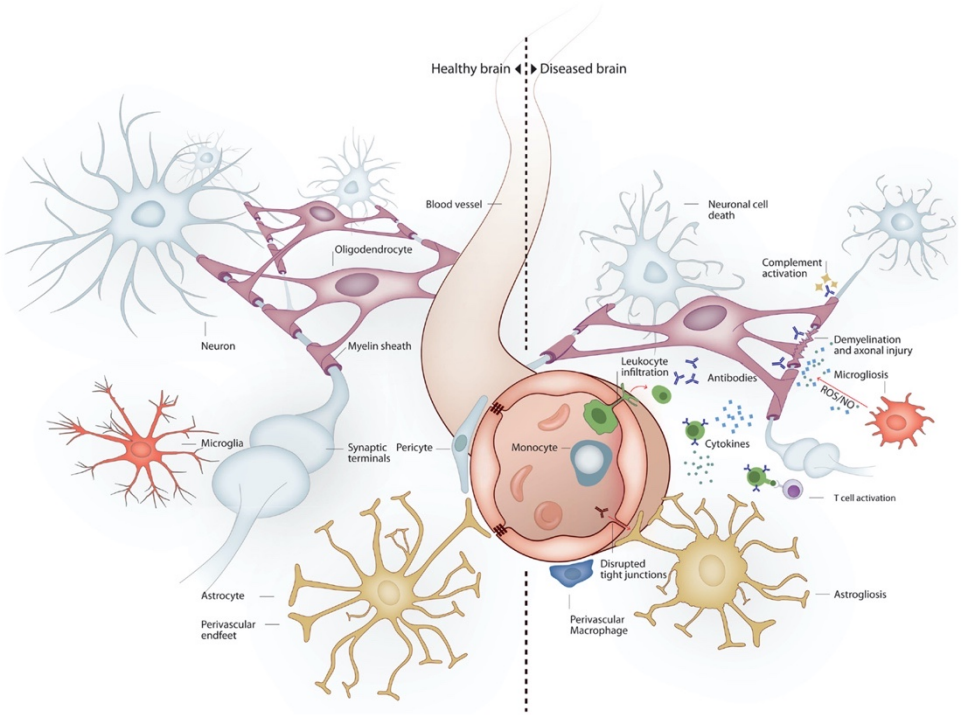


Figure 5 | Brain milieu changes in response to chronic systemic inflammation. The main cells present in the brain are neurons, oligodendrocytes, astrocytes, and microglia. Under healthy conditions (left), neurons connect to each other through long axonal processes with synapses. Oligodendrocytes support axons with myelin sheaths. Astrocytes interact with blood vessels to form the blood-brain barrier and support neuronal synapses. Microglia form long processes that surveillance the brain and phagocytose apoptotic cells and prune inactive synapses without induction of inflammation. Under chronic inflammatory conditions (right), several mechanisms can lead to neurodegeneration. Peripheral immune cells and inflammatory molecules traverse the blood-brain barrier exerting direct and indirect neuronal cytotoxicity. Oligodendroglial myelin sheaths can be affected leading to axonal degeneration. An influx in astrocytes can lead to reduced blood-brain barrier and synaptic maintenance. An influx in microglia can lead to a pro-inflammatory phenotype with reduced phagocytic and tissue maintenance functions. Adapted from "*Systemic inflammation and the brain: novel roles of genetic, molecular, and environmental cues as drivers of neurodegeneration*", by R. Sankowski, et al., 2015, Frontiers in Cellular Neuroscience, 9, p. 4. © 2015 by Frontiers, Inc. Adapted with permission.

1.4.1 Measurements of inflammation

The most common methods to measure inflammation directly are to quantify protein biomarkers (e.g., C-reactive protein (CRP), proinflammatory cytokines) or the molecular biomarkers that regulate them (e.g., gene expression). Gene expression is the process by which information from a gene is used to direct the assembly of a protein (Figure 6). It is the fundamental process by which the genotype (i.e., genetic characteristic) gives rise to the phenotype (i.e., physical characteristic). Gene expression levels—the quantity of messenger RNA (mRNA) transcripts—are not static; these levels can be downregulated or upregulated dependent on genetic determinants, epigenetic modifications, and environmental factors, including stress and trauma exposure.[104] Gene expression analysis is a laboratory technique that allows for the unbiased identification of dysregulated gene expression levels across the whole transcriptome in a target population.[105] Dysregulated mRNA transcripts can then be

aggregated into networks and pathways based on their biological function (e.g., immune response pathway). However, a prominent problem of using gene expression analysis to measure inflammation in populations with psychiatric disorders is that individual gene expression differences are usually modest, which makes it difficult to distinguish true alterations from normal human variation. Some reasons for this include: mRNA transcripts can encode for several different proteins and the levels of mRNA transcripts do not always correspond with the levels of proteins they encode for.[106] This makes protein biomarker analysis an important downstream complement to measure inflammation. While direct sampling of the brain is not readily assessable in living humans, protein biomarkers assessed in peripheral blood can provide a surrogate indicator of neuroinflammation in the brain.[107] However, conclusions must be tempered since inflammation assessed in peripheral blood does not always correlate with cerebral spinal fluid levels,[108] and peripheral blood levels may be subject to a blunted circadian rhythm.[109,110] Nonetheless, the emerging role of biomarkers in psychiatry marks a retreat from a purely symptom-based diagnostic system, which is vulnerable to interpretation and subjective reporting. Moreover, the identification of biological systems associated with risk and symptom severity holds promise for the development of diagnostic tests, prognostic indicators, prophylactics, and treatments for trauma-exposed populations.[111]

Transcriptome: all RNA transcripts, including coding and non-coding

Transcriptomics: the study of the transcriptome and its function

Transcription: the process by which information in DNA is copied into an mRNA transcript

Translation: the process by which protein is synthesized from information contained in an mRNA transcript

Gene expression analysis: the quantification of mRNA transcripts

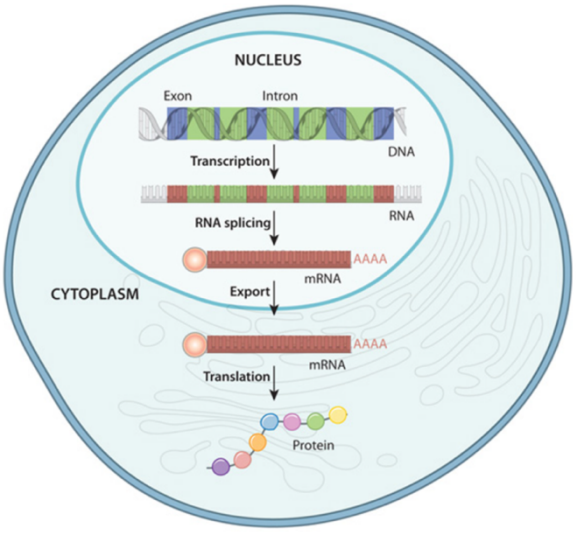


Figure 6 | An overview of the flow of information from gene to protein in a human cell. First, both protein-coding and noncoding regions of DNA are transcribed into RNA. Some regions are removed (e.g., introns) during initial RNA processing. The remaining exons are then spliced together, and the spliced messenger RNA (mRNA) molecule (red) is prepared for export out of the nucleus through the addition of an endcap (sphere) and a polyA tail. Once in the cytoplasm, the mRNA can be used to construct a protein. Adapted from "*Essentials of Cell Biology"*, by C.M. O'Connor & J. U. Adams, 2010, NPG Education, Cambridge, MA. © 2010 by Nature Education. Adapted with permission.

1.4.2 Link between inflammation and posttraumatic stress

When stress is chronic, the immune system can become dysregulated, and proinflammatory cytokines [e.g., interleukin-1beta (IL-1β), interleukin-6 (IL-6), interferon-gamma (IFNγ), and tumor necrosis factor-alpha (TNF-α)] can increase in the periphery. These peripheral inflammatory mediators can communicate with the brain.[23] Additionally, chronic stress can activate microglia (resident cells within the brain) and cause the release of inflammatory mediators, including proinflammatory cytokines, within the brain.[112] Taken together, the consequences of these two processes may increase neuroinflammation and putatively contribute to the onset or severity of psychiatric symptoms.[113,114] A 2015 meta-analysis (*N* = 1348), indicated that individuals with PTSD had increased blood levels of IL-1β, IL-6, and IFNγ compared with controls.[115] Of note, these meta-analytic findings remained significant even after excluding studies that included participants with comorbid major depressive disorder from the analysis.[115] Moreover, when studies with medication naive participants were exclusively analyzed, these same protein biomarkers plus levels of TNF-α were higher in individuals with PTSD compared to controls.[115] This is particularly important given that major depressive disorder and medication use commonly occur with PTSD and are also associated with changes in levels of inflammation.[116] A second 2018 meta-analysis (*N* = 1077), which solely included studies with clinician diagnosed participants, reported that individuals with anxiety disorders, obsessive-compulsive disorder, and PTSD had elevated blood levels of IL-1β, IL-6, and TNF-α compared to healthy controls.[117] Despite these findings, the observational design of the included studies restricted the meta-analyses from determining causal associations.[118] Several studies also suggest molecular biomarkers that regulate the proinflammatory milieu in PTSD. For example, a mega-analysis of blood transcriptome studies (*N* = 511) found that the innate immune, cytokine, and type I interferon signaling pathways were associated with PTSD across all three case-control groups: men exposed to combat traumas, men exposed to interpersonal traumas, and women exposed to interpersonal traumas.[119] Nevertheless, further work is needed to determine the extent to which these biomarkers can serve as specific and sensitive markers of posttraumatic stress symptoms.

1.4.3 Link between inflammation and sleep

The existence of a bidirectional relationship between sleep and the immune system is well-recognized—sleep helps regulate important immune functions, and the immune system regulates certain aspects of sleep.[120] Although sleep plays an essential role in regulating the innate immune system, the evidence linking sleep to systemic inflammation is inconsistent.

This variability could be partly explained by the diverse parameters used in clinical trials to assess sleep quality (e.g., objectively measured, clinician assessed, or self-reported), as well as the duration of sleep deprivation (e.g., years, days, one night, or part of a night). In this respect, a recent meta-analysis (N > 50,000) of blood-based biomarkers found that subjectively measured sleep duration (i.e., clinician assessed or self-reported) was not associated with levels of CRP (n = 3490) or IL-6 (n = 2084).[121] There was also no association between objectively measured sleep duration and levels of CRP (n = 1550); however, objectively measured short sleep durations were correlated with higher levels of IL-6 (n = 489).[121] In studies that used a combination of objective and subjective measures, short sleep durations were correlated with higher levels of CRP (n = 5040) but were not associated with levels of IL-6 (n = 2573) or TNF-α (n = 157).[121] A meta-analysis of experimental sleep deprivation studies found no evidence of altered levels of CRP (n = 218), IL-6 (n = 263), or TNF-α (n = 109) following sleep restriction over 2 to 12 consecutive days, for one night, or for part of a night—sleep was reduced from 8 hours to 4 hours.[121] Adding to these inconsistencies, partial sleep deprivation was shown to upregulate transcription of *IL-6* and *TNF-α* mRNAs, while improved sleep quality following standardized sleep treatment was found to downregulate transcription of inflammatory-related mRNAs.[122-124] This highlights the variations of findings and the importance of utilizing a combination of objective and subjective sleep measures and analyzing both protein and molecular biomarkers to capture a more comprehensive picture of the relationship between sleep disturbance and inflammation.

1.5 THESIS RATIONALE

In 2017 the American Psychological Association published their most recent clinical practice guidelines for the treatment of adults with PTSD.[125] Based on the strength of scientific evidence and patient preference, the guidelines recommended trauma-focused psychotherapy over pharmacotherapy as a first-line treatment for PTSD.[125] At the heart of trauma-focused psychotherapy is imaginal and *in vivo* exposure to trauma reminders. This therapy is based on the premise that PTSD emerges due to the development of a fear-based memory.[126] In theory, effective therapy involves correcting the pathological elements of the fear-based memory by first reactivating the memory so it is labile and then introducing new information that corrects the existing pathological components.[127] While trauma-focused psychotherapies are efficacious for some patients, these treatments tend to be less effective in military populations.[128] For example, between 30 to 51% of military service members do not demonstrate clinically meaningful symptom reduction, and remission rates are as low as

17 to 27%.[129] Even when trauma-focused psychotherapy is combined with medication, no additional improvements are found.[130,131] Thus, there is a need for improving existing PTSD treatments in military populations, with a better understanding of patient preference, disease mechanisms, and biomarkers underlying treatment response. This next section will briefly review the inextricable link between stress, sleep, and inflammation and alternative interventions that target these systems will be discussed.

1.5.1 Link between stress, sleep, and inflammation

Most people experience some type of adversity or trauma in their lifetime. The body is designed to activate the stress response system at low levels in response to these types of events.[132] However, when there is sustained engagement of the stress response system, due to persistent psychosocial stress, or ruminating over past or future stressors, levels of inflammation can increase in the periphery and in the brain (Figure 7).[133,134] Theoretically, this increased inflammation, may induce a biphasic shift in sleep patterns—initially, sleep continuity is increased in an effort to restore homeostasis (not illustrated); then later, sleep continuity is decreased paired with an increase in sleep architecture disturbances.[135,136] Due to the bi-directional relationship between sleep and the immune system, prolonged sleep disturbance may shift molecular and protein profiles to those with increased inflammatory expression.[136] Figure 7 illustrates how this chronic inflammatory state might contribute to the maintenance of PTSD (including its resistance to treatments) and explain its high comorbidity with other mental and physical health conditions.[137] Furthermore, PTSD triggers may create a vicious feedback cycle and compound symptoms. Therefore, interventions that target the reciprocal sleep–immune relationship may have the potential to redirect this askew inflammatory expression and reduce stress-related symptoms.

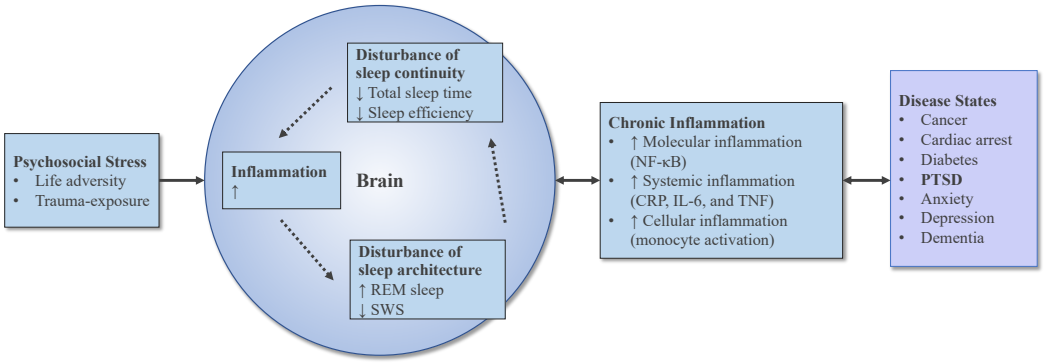


Figure 7 | Proposed model of how psychosocial stress may contribute to disease states. CRP, C-reactive protein; IL, interleukin; NF-κB, nuclear factor-kappa B; PTSD, posttraumatic stress disorder; REM, rapid eye movement; SWS, slow-wave sleep; TNF, tumor necrosis factor.

1.5.2 Sleep-focused treatments

As new evidence points to sleep disturbance as a predictor of PTSD onset and relapse, sleep-focused treatments may be an effective and systemic means to mitigate posttraumatic stress symptoms, as well as the putative underlying inflammation. Sleep-focused treatments may also be preferred to trauma-focused psychotherapies due to the stigma associated with mental health issues, especially by military personnel.[138] Cognitive behavioral therapy for insomnia (CBT-I) is a first-line treatment for insomnia, which targets maladaptive sleep-related thought patterns and behaviors.[139] A 2015 meta-analysis (N = 2189) reported that 36% of insomnia participants who received CBT-I were in remission from insomnia and the CBT-I had similar positive effects on co-occurring mental and physical health symptoms.[140] Although these results are promising, a sizable percentage of the population still remained symptomatic. This highlights the need to investigate the efficacy of alternative interventions that target stress, sleep, and inflammation, directly or indirectly.

1.5.3 Mindfulness meditation programs

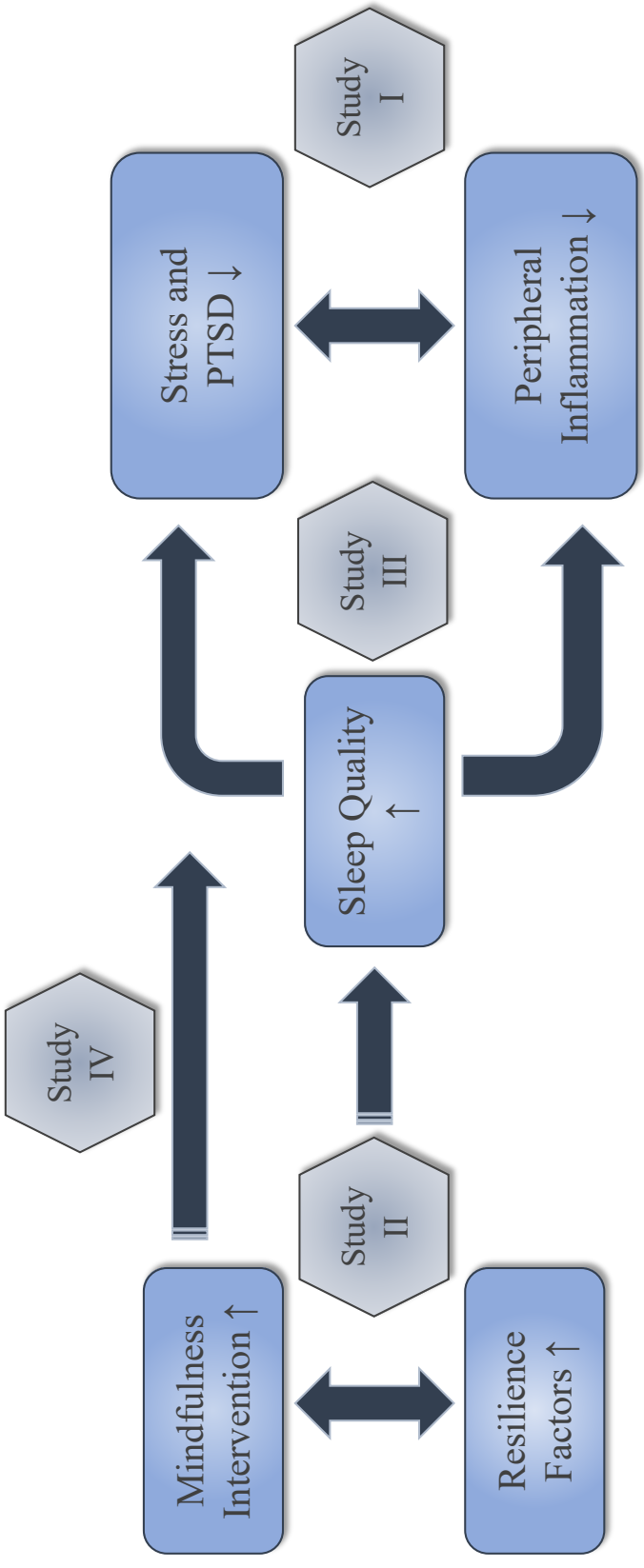
Mindfulness is a type of meditation with roots in an ancient Buddhist practice called Vipassanā.[141] In a research context, mindfulness is typically defined as paying attention in a particular way: on purpose, in the present moment, and non-judgmentally.[142] In recent years, mindfulness meditation has gained interest as a transdiagnostic intervention linked to improvements in various mental and physical health outcomes.[143] In clinical trials, mindfulness-based stress reduction (MBSR) is the most popular mindfulness-based intervention tested; it includes eight weekly, 2.5-hour in-class sessions with daily homework exercises. Modified courses have since been created, such as mindfulness-based cognitive therapy (MBCT), a hybrid of MBSR and CBT. Mindfulness meditation is hypothesized to target brain regions implicated in cognition and emotional regulation.[144] It has been shown to decrease emotional reactivity, diminish ruminative thoughts, and facilitate the impartial reappraisal of salient events.[145-148] Systematic reviews have reported tentative evidence that mindfulness meditation reduces physiological markers of stress, blood levels of CRP, and expression levels of NF-κB.[149,150] Several meta-analyses have investigated the effect of mindfulness meditation on sleep quality; however, the results have been inconclusive.[151-153] Of these, the most robust meta-analysis reported insufficient evidence to draw any conclusions due to the limited number of available trials (n = 8) at the time of publication in 2014.[153] Since then, there has been an exponential growth in mindfulness research, thus its potential effect on sleep quality warrants another review.

1.5.4 Integrative medicine programs

Integrative medicine programs include mindfulness practices, but also make use of other conventional and alternative evidence-based methods to support the body's innate healing response using the least invasive methods as appropriate (Figure 8).[154] Integrative medicine is patient-centered care that goes beyond the treatment of the initial symptoms and aims to address all potential causes and consequences of disease.[155] This has important implications for military service members who often present with chronic conditions, including the signature polytrauma triad of chronic pain, mTBI, and PTSD.[156] Several theoretical models of integrative medicine have been proposed depicting putative mechanisms of action. The most commonly-cited include the emphasis on the mindbody connection and the patient-practitioner alliance and their collaboration in designing and implementing a comprehensive treatment plan.[157] Unlike more robust clinical trials, which attempt to control for extraneous factors (by randomizing and blinding etc.), integrative medicine leverages a patient's preferences, perspectives, and beliefs, as well as the patient-practitioner relationship to enhance the effect of the intervention.[158] As such, the outcome does not occur in isolation from the extraneous factors, but in synergy with them. Investigation of the program's effectiveness typically treats the program as a synergistic bundle without dissecting the program components.[158]

Figure 8 | The Wheel of Health - The Osher Center for Integrative Medicine, Vanderbilt University Medical Center. The Wheel of Health illustrates the integrative approach to healing and wellness. It is patient-centered and complements traditional care with additional therapies that are backed by scientific evidence to improve health and promote healing. © 2015 Vanderbilt University Medical Center. Reprinted with permission.

Model depicting the inter-relationship of all four studies.



2 STUDY AIMS

The general aim of this thesis was to examine the inextricable link between stress, sleep, and inflammation, and how gaining a better understanding of these interconnected systems could be harnessed to develop enhanced treatments for populations with stress-related symptoms and disorders. The aims for each study follow.

STUDY I investigated the effect of 4 to 8 weeks of CBT-I and automatic positive airway pressure (APAP) therapy on posttraumatic stress symptoms, as well as the gene expression pathways that may mediate this effect in sleep disturbed military service members with and without PTSD. The secondary aim was to determine the gene expression profiles that were associated with each of the three PTSD symptom clusters: intrusions, avoidance/numbing, and arousal symptoms.

STUDY II was a meta-analysis that assessed the effect of mindfulness meditation on sleep quality outcomes when compared to specific active controls (i.e., evidence-based sleep treatments) and nonspecific active controls (i.e., time/attention matched placebo controls) in sleep disturbed adults with a mental and physical health condition. The secondary aims were to assess for a long-term effect at 5- to 12-month follow-up and assess for a dose-response relationship between in-class meditation hours and sleep quality improvements.

STUDY III investigated if sleep quality improvements, following a 4-week mindfulness-based integrative medicine program, were associated with reductions in posttraumatic stress (primary outcome), anxiety, depression, and postconcussion symptoms in sleep disturbed military service members with mTBI. The secondary aim was to determine if sleep quality improvements were associated with decreases in plasma levels of IL-6, IL-10, and TNF-α.

STUDY IV investigated the effect of a brief 5-week (7.5-hour) mindfulness-based program on perceived stress symptoms in moderately stressed healthcare professionals. Secondary outcomes included burnout, anxiety, positive and negative affect, state and trait mindfulness, and self-care.

In the great drama of existence,
We are audience and actors at the same time.

— Niels Bohr

3 METHODS

This thesis included a range of methods and a comprehensive account can be found in the corresponding manuscripts (see Chapter 9. Appendix). Sleep-focused interventions were complemented with mechanistic studies of molecular and protein biomarkers. Since participant enrollment for this thesis began in 2013, the DSM-IV definition of PTSD is consistently used throughout all relevant studies. In Study I and Study III a combination of polysomnography, clinician assessed, and self-report measures were used to determine sleep disturbance. In Study II both actigraphy and self-report measures of sleep quality were used, but due to the insufficient number of studies that reported on actigraphy data (*n* = 2), only the self-report measures of sleep quality were included in the meta-analysis. An overview of the study design, population, intervention, outcomes, and main statistical test for each study is presented in Table 2.

Table 2 | Overview of study characteristics.

	STUDY I	STUDY II	STUDY III	STUDY IV
Study Design	Case-Control	Meta-Analysis	Observational	Randomized Clinical Trial
Population	Sleep disturbed military service members with or without PTSD	Sleep disturbed adults with a mental and physical health condition	Sleep disturbed military service members with mTBI	Moderately stressed healthcare professionals
Groups	PTSD = 39 No PTSD = 27	Meditation = 917 Control = 865	Intervention = 93	Meditation = 43 Control = 35
Intervention	CBT-I and APAP	Mindfulness meditation	Mindfulness-based integrative medicine	Brief mindfulness meditation
Duration	4 to 8 weeks	2 to 16 weeks	4 weeks	5 weeks
Main Outcome	Posttraumatic stress	Sleep quality	Posttraumatic stress	Perceived stress
Biomarkers	Genome-wide gene expression	Not included	Plasma IL-6, IL-10, and TNF-α	Not included
Statistical Test	ANOVA	Hedges' *g*	Linear regression	GLMM

ANOVA, analysis of variance; APAP, automatic positive airway pressure; CBT-I, cognitive behavioral therapy for insomnia; GLMM, generalized linear mixed models; IL, interleukin; PTSD, posttraumatic stress disorder; mTBI, mild traumatic brain injury; TNF-α, tumor necrosis factor-alpha

3.1 STUDY I: METHODS

Study Design

This was a case-control study that investigated the effect of 4 to 8 weeks of CBT-I and APAP therapy on posttraumatic stress symptoms, as well as the gene expression pathways that may mediate this effect in sleep disturbed military service members with and without PTSD. At baseline, participants diagnosed with insomnia received 4 to 8 biweekly sessions of CBT-I, and participants diagnosed with obstructive sleep apnea received APAP therapy. Baseline (week 1) and follow-up (week 12) visits included a battery of self-report questionnaires and blood draws.

Participants

Participants were characterized using the PTSD Checklist—Military Version (PCL-M).[159] Participants with a baseline PCL-M score ≥ 50 formed the PTSD group (n = 39) and participants with a baseline PCL-M score ≤ 25 formed the control group (n = 27); these are the proposed cut-points for military prevalence.[160] At follow-up, the PTSD group was further divided into two groups: PTSD improved (n = 12; PCL-M change score reduction ≤ -5) and PTSD not-improved (n = 11; PCL-M change score ≥ 0).[161]

Biomarker Acquisition

Blood was collected into PAXgene tubes (PreAnalytiX Inc.; Hombrechtikon, Switzerland) and stored at −80 °C until RNA extraction using the PAXgene Blood RNA Kit. Samples were reverse transcribed using the GeneChip 3′ IVT Expression Kit and hybridized to Affymetrix HG-U133 Plus 2.0 microarrays (Affymetrix Inc.; Santa Clara, CA, USA).

Statistical Analysis

Analysis of variance (ANOVA) was used to compare baseline gene expression data between groups and paired t-tests were used to compare baseline to follow-up gene expression changes within groups. PCL-M symptom cluster subgroups were created using equal 33.33 percentile cut-points, whereby participants who endorsed the highest symptoms (high 1/3) were compared with participants who endorsed the lowest symptoms (low 1/3). Data were analyzed at a ± 2.0-fold change magnitude at a false discovery rate ≤ 0.05 using Partek Genomics Suite 6.6 (Partek Inc.; St. Louis, MO, USA). This criterion was determined by a power analysis indicating that the given ANOVA at a ± 2.0-fold change magnitude, would require 35 samples total for a beta of 0.80.[162,163] All gene lists were then uploaded into the Ingenuity Pathway Analysis (QIAGEN; Redwood City, CA, USA).

3.2 STUDY II: METHODS

Systematic Search

This was a meta-analysis that assessed the effect of mindfulness meditation on sleep quality outcomes when compared to specific active controls (i.e., evidence-based sleep treatments) and nonspecific active controls (i.e., time/attention matched placebo controls) in sleep disturbed adults with a mental or physical health condition. PubMed, EBSCO, Embase, and The Cochrane Library databases were searched for articles through May 2018, with no start date restriction. For search terms, two main subject-heading domains were combined with the AND operator: one to designate the intervention (meditation, mindfulness, MBSR, MBCT, or Vipassanā) and the second to designate the outcome (sleep or insomnia).

Inclusion and Exclusion Criteria

Randomized controlled trials were included in the meta-analysis that enrolled populations of sleep disturbed adults and employed a mindfulness meditation intervention with sleep quality assessments at baseline and postintervention. Evidence-based sleep treatments were determined by an American Academy of Sleep Medicine 2006 report and updated with a 2015 meta-analysis reporting medium to large effects of physical activity on subjective measures of sleep quality.[164,165] Validated sleep measures included both objective and subjective measures. Trials were excluded that compared mindfulness meditation to an experimental sleep treatment or compared novice meditators to experienced meditators. All other populations with clinically significant sleep disturbance, excluding children and adolescents, were eligible (Table 3).

The Strength of the Body of Evidence

To determine the strength of the body of evidence, three investigators graded the strength of evidence for each outcome using the grading scheme recommended by the Methods Guide for Conducting Comparative Effectiveness Reviews.[166] In assigning evidence grades, four domains were considered: risk of bias, directness of outcome measures, consistency of results, and precision of results. Evidence was classified into the following four categories: (1) high confidence that the estimate of effect lies close to the true effect, and further studies would not change the conclusion; (2) moderate confidence that the estimate of effect lies close to the true effect, and findings are likely to be stable, but some doubt remains; (3) low confidence that the estimate of effect lies close to the true effect, and that additional evidence is needed; and (4) insufficient or no evidence to estimate an effect.[166]

Table 3 | Detailed inclusion and exclusion summary.

	Inclusion	Exclusion
POPULATION	Adult populations with clinically significant sleep disturbance (i.e., insomnia diagnosis or met symptom severity threshold defined by sleep quality questionnaires)	Children, adolescents, and experienced meditators
INTERVENTION	In-person, structured mindfulness meditation (e.g., mindfulness-based stress reduction)	Mantra-based meditation, movement-based therapies like tai chi, and internet administration
COMPARATOR	Specific active controls: evidence-based sleep treatments Nonspecific active controls: time /attention-matched interventions	Waitlist or usual care controls
OUTCOME	Assessment of a baseline and postintervention validated objective or subjective measure of sleep quality	No validated measure of sleep quality or only a baseline measurement
STUDY DESIGN	Randomized controlled trials	Non-randomized controlled trials
OTHER	All languages and dates through May 2018	Abstracts, reviews, and non-published trials, as well as duplicate participant samples

Adapted from "*The effect of mindfulness meditation on sleep quality: a systematic review and meta-analysis of randomized controlled trials*", by H. L. Rusch, et al., 2018, Annals of the New York Academy of Sciences, 1445(1), p. 7. © 2018 by John Wiley & Sons, Inc. Adapted with permission.

Outcome Measures

Objective measures of sleep quality included actigraphy. Subjective measures with established validity included the Insomnia Severity Index (ISI), the Medical Outcomes Study—Sleep Scale (MOS-SS), and the Pittsburgh Sleep Quality Index (PSQI).[167-169] Due to the high overlap in content validity between the three sleep quality scales, they were pooled in the meta-analysis.

Statistical Analysis

Quantitative data were analyzed with the Cochrane Collaborative Review Manager Software (RevMan 5.3).[170] Since sleep quality measures differed between trials (e.g., ISI, MOS-SS, and PSQI), the between-group standardized mean difference was used as the summary effect estimate of sleep quality and was calculated as Hedges' *g*. A meta-analysis was used to estimate the long-term effects of trials with a follow-up assessment between 5 to 12 months from baseline. To test for relative efficacy, all meta-analyses were stratified by control type (i.e., specific active control or nonspecific active control). Spearman's correlation was used to test for a dose-response relationship between in-class meditation hours and standardized sleep quality change scores.

3.3 STUDY III: METHODS

Study Design

This was an observational study that investigated if sleep quality improvements, following a 4-week mindfulness-based integrative medicine program, were associated with reductions in neurobehavioral symptoms and decreases in levels of inflammatory biomarkers in sleep disturbed military service members with mTBI. Participants underwent a 4-week intensive outpatient program that combines conventional rehabilitation therapies with complementary wellness interventions. The wellness interventions included acupuncture, biofeedback, creative arts therapy, and mindfulness practices (e.g., breathing, meditation, yoga). All interventions were administered by credentialed providers. Baseline (week 1) and follow-up (week 4) visits included a battery of self-report questionnaires and blood draws.

Participants

A total of 98 military service members consented for this study and 93 met eligibility criteria. Inclusion criteria included active military service members, 18 years of age or older, who sustained combat- or mission-related mTBI, with sleep disturbance. Exclusion criteria included a positive urine pregnancy test (for individuals with childbearing potential) and an inability to consent. The Ohio State University, Traumatic Brain Injury Identification Method (OSU TBI-ID) was used to diagnose mTBI, and the Pittsburgh Sleep Quality Index (PSQI) was used to measure baseline and follow-up sleep quality, and screen for self-reported sleep disturbance over the prior month.[167,171] To provide the maximum sensitivity (0.90) and specificity (0.87), a total PSQI score of six or more indicated a positive screen for disturbed sleep. Five military service members did not have sleep disturbance based on a PSQI score of six or more and were considered ineligible.

Outcome Measures

The primary outcome, the PTSD Checklist—Military Version (PCL-M), was used to assess self-reported posttraumatic stress symptoms.[159] Secondary self-report outcomes included the General Anxiety Disorder 7-Item (GAD-7), to assess anxiety; the Patient Health Questionnaire 9-Item (PHQ-9), to assess depression; and the Neurobehavioral Symptom Inventory 22-Item (NSI-22), to assess postconcussion symptoms.[172-174]

Biomarker Acquisition

Blood samples were collected through routine venipuncture into ethylenediaminetetraacetic acid tubes then aliquoted on ice into cryovials. The cryovials were stored upright in a

−80°C freezer until assayed. The Single Molecule Array HD-1 Analyzer (SIMOA; Quanterix, Billerica, MA) was used to measure plasma levels of inflammatory biomarkers using manufacturer's instructions, consumables, and reagents. The SIMOA's digital technology increases sensitivity by 1,000-fold on average over conventional immunoassays.[175] Multiplex technology simultaneously quantified IL-6, IL-10, and TNF-α in one kit. Samples were batched assayed to minimize variability, and standards and controls were run with each batch to confirm reliability. All samples were analyzed in duplicate and all values were above the limit of detection. Samples were rerun when coefficients of variance exceeded 20%. Data were not used if coefficients of variance were above 20% after rerun, and intra-assay or inter-assay performance was below 20%.

Statistical Analysis

Statistical analyses were performed using the statistical software R (Version 3.3.3 for Mac; R Core Team, Vienna, Austria) and SPSS (Version 26 for Mac; IBM Corp, Chicago, IL). Categorical variables were described in frequencies and percentages and compared with chi-square tests, while continuous variables were described in means and standard deviations and compared with unpaired or paired *t*-tests as appropriate. Results were similar with and without the outliers, so all available data were included. All tests were two-sided and a *p*-value less than 0.05 was considered to be statistically significant. Linear regression models were used to assess the association between change in sleep quality (from baseline to follow-up) and change in neurobehavioral symptom severity and change in levels of inflammatory biomarkers over this same 4-week period. For example, the posttraumatic stress regression model included PSQI_Change as the predictor variable and PCL-M_Change as the dependent variable, while also adjusting for PSQI at baseline, PCL-M at baseline, age, body mass index, race, tobacco use, and alcohol use. In a subgroup analysis, we conducted repeated-measures ANOVAs to investigate group differences between participants with improved sleep quality and those with declined sleep quality on changes in dependent variables found to be significantly associated with sleep from the main analysis. A three-point reduction on the PSQI is considered the minimal clinically important difference and was used to distinguish participants with improved sleep quality (PSQI change score ≤ -3) from those with declined sleep quality (PSQI change score ≥ 1).[176] Lastly, missing data patterns (e.g., participants who missed follow-up assessments due to lack of time) were examined with sensitivity analyses, based on imputation, for their impact on model inferences.

3.4 STUDY IV: METHODS

Study Design

This was a randomized clinical trial that investigated the effect of a brief 5-week (7.5-hour) mindfulness-based program on perceived stress symptoms in moderately stressed healthcare professionals. Participants were randomized into the mindfulness-based self-care (MBSC) group or the life-as-usual control group. The MBSC group attended five 1.5-hour mindfulness sessions at the National Institutes of Health, Clinical Center during work hours. Mindfulness exercises included mindful breathing, body scan, mindful walking, mindful movements, mindful eating, and loving-kindness meditation. Daily at-home mindfulness practice was strongly encouraged. Baseline (week 1) and postintervention (week 5) visits included a battery of self-report questionnaires for both groups, as well as a follow-up (week 13) visit for the MBSC group only to test for a maintenance effect.

Participants

Participation was open to all National Institutes of Health employees, contractors, and training fellows. Individuals with psychiatric and medical conditions were advised to consult with their healthcare providers before enrollment. Two participants in each group declined to participate after randomization, before the start of the study and did not provide baseline data. Thus, the modified intent-to-treat analyses included 43 meditation participants and 35 controls (Figure 9).

Outcomes

The primary outcome, the Perceived Stress Scale 10-Item (PSS-10) was used to assess self-reported stress.[177] Secondary self-report outcomes included the Visual Analog Scale—Anxiety (VAS-A) to assess anxiety, the Maslach Burnout Inventory 2-Item (MBI-2) to assess burnout, the Positive and Negative Affect Schedule (PANAS) to assess positive and negative affect, the Mindful Attention Awareness Scale—Trait and State (MAAS-T and MAAS-S) to assess trait and state mindfulness, and the Mindful Self-Care Scale—General (MSCS-G) to assess mindfulness self-care practices.[178-182]

Statistical Analysis

Sample size was determined by calculating the power to detect a mean change score of 1-point in perceived stress (PSS-10) between the meditation and control groups from baseline to postintervention.[183] Estimates assumed a moderate-to-strong correlation (rho = 0.5) in the repeated measures, equal group allocation, two-sided alpha of 0.05, beta of 0.90, and an

attrition rate of 10 to 15%, which yielded 33 participants per group. Generalized linear mixed modeling for repeated measures compared postintervention and follow-up measures between and within the meditation and control groups, as applicable. Mixed models are the recommended statistical method for analyzing data collected at repeated time points.[184] This method has the advantage of making use of all available data, accounting for within-subject correlations between repeated measurements, and implicitly accounting for data missing at random. Post-hoc pairwise comparisons were adjusted for multiple comparisons using the Bonferroni method and reported *p*-values were corrected for multiplicity. Effect sizes were bias corrected with Cohen's *d* and Hedges' *g*.

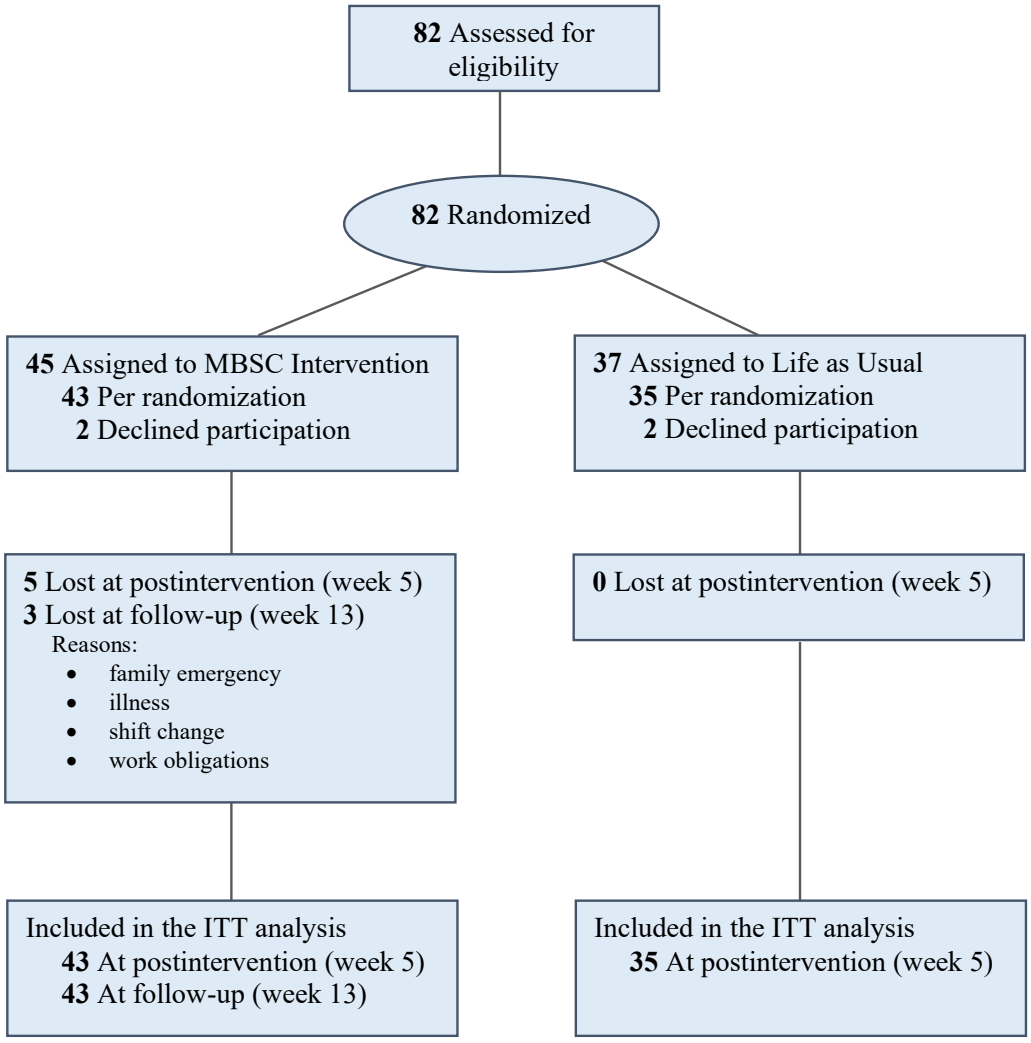


Figure 9 | CONSORT flow diagram. The study flow starting at enrollment and continuing through randomization, postintervention, and follow-up. MBSC, mindfulness-based self-care; ITT, intent-to-treat. Adapted from "*Effect of a brief mindfulness-based program on stress in health care professionals at a US biomedical research hospital: A randomized clinical trial*", by R. Ameli, et al., 2020, JAMA Open Network, 3(8). © 2020 by the American Medical Association. Adapted with permission.

4 RESULTS

Each study included a series of main and sub-analyses. This section briefly describes the results for each study; a comprehensive account can be found in the corresponding manuscripts (see Chapter 9. Appendix). An overview of the main findings for each study is presented in Table 4.

Table 4 | Overview of study results.

STUDY I	Posttraumatic stress symptom reduction, following 4 to 8 weeks of CBT-I and APAP therapy, was linked to downregulated cell-mediated immune response genes and immune cell trafficking genes.[a]
STUDY II	Mindfulness meditation is an effective treatment to improve sleep quality in adult populations with various mental and physical health conditions.
STUDY III	Sleep quality improvements, following a 4-week mindfulness-based integrative medicine program, were linked to reductions in posttraumatic stress symptoms, but not to decreases in inflammation.
STUDY IV	A brief 5-week (7.5-hour) mindfulness-based program reduced perceived stress in moderately stressed healthcare professionals.

APAP, automatic positive airway pressure; CBT-I, cognitive behavioral therapy for insomnia

[a] Downregulation is the process by which a cell decreases the quantity of mRNA transcripts associated with a particular gene. Upregulation is the complementary process. The terms underexpression and overexpression are used synonymously.

4.1 STUDY I: RESULTS

Study I investigated the effect of 4 to 8 weeks of CBT-I and APAP therapy on posttraumatic stress symptoms, as well as the gene expression pathways that may mediate this effect in sleep disturbed military service members with and without PTSD. The baseline demographic and clinical characteristics for the PTSD and control groups are presented in Table 5.

Table 5 | Baseline demographic and clinical characteristics.

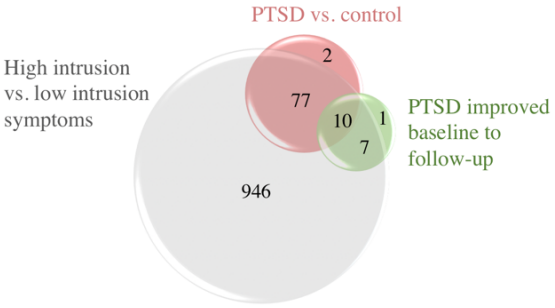
	PTSD (*n* = 39)	Controls (*n*= 27)	x^2 / *t*	*p*-value
Age: *n* (%)				
18 – 35 years	27 (69.2)	14 (51.9)	2.048	0.152
36 – 55 years	12 (30.8)	13 (48.1)		
Sex: *n* (%)				
Male	37 (94.9)	26 (96.3)	0.075	>0.999
Female	2 (5.1)	1 (3.7)		
Race: *n* (%)				
White	25 (64.1)	20 (74.1)	0.731	0.392
Non-white	14 (35.9)	7 (25.9)		
Education: *n* (%)				
No college	16 (42.1)	10 (37.0)	0.169	0.681
Some college	22 (57.9)	17 (63.0)		
Body mass index: *mean (SD)*	29.8 (4.4)	29.6 (3.9)	-0.196	0.846
Complex insomnia: *n* (%)	18 (46.2)	3 (11.1)	9.031	**0.003**
mTBI diagnosis: *n* (%)	29 (74.4)	1 (3.7)	32.125	**< 0.001**
PTSD severity: *mean (SD*)	61.3 (7.7)	21.7 (2.7)	-25.552	**< 0.001**

mTBI, mild traumatic brain injury; PTSD, posttraumatic stress disorder

The first gene expression analysis indicated that, at baseline, 89 genes were differentially expressed between participants with and without PTSD. Most of these genes (94%) were upregulated in the PTSD group. The next analysis compared the gene expression differences, also at baseline, between participants who endorsed high vs. low symptom severity among the three PTSD symptom clusters (see Table 1 for review). The purpose of this analysis was first to see if certain genes were associated with specific posttraumatic stress symptoms and secondly to see if a more robust gene expression profile would emerge for the intrusion symptom cluster (e.g., reexperiencing, nightmares, and flashbacks) since these are hallmark features of PTSD. As such, the gene expression analysis indicated that 1040 genes were differentially expressed between participants with high vs. low intrusion symptoms. Most of these genes (98%) were upregulated in the high intrusion symptom group. There were no differentially expressed genes between participants with high vs. low avoidance/numbing symptoms or with high vs. low arousal symptoms.

Of the PTSD participants, 22.6% had clinically meaningful improvements (change score reduction ≤ -10).[b] The final analysis sought to determine which genes were associated with symptom improvement in this group. Herein, the gene expression analysis indicated that 18 genes were differentially expressed in the PTSD group with improved symptoms from baseline to follow-up. All 18 genes were downregulated at follow-up and ten of these genes intersected with the PTSD vs. control differentially expressed gene list and the high vs. low intrusion symptom differentially expressed gene list, but with an inverse relationship (Figure 10).

A



B

Gene Symbol	PTSD	High Intrusion	PTSD Improved
C5orf24	2.45249	3.15414	-2.13559
RBAK	2.58129	2.50236	-2.13466
CREBZF	2.25318	3.03567	-2.10773
CD69	2.31687	3.53799	-2.09885
PMAIP1	2.32123	3.09929	-2.05361
AGL	2.03686	2.77836	-2.04094
ZNF644	2.20669	3.42249	-2.03622
ANKRD13C	2.10257	2.78078	-2.03553
ESCO1	2.10389	2.33777	-2.01837
ZCCHC10	2.49841	3.62007	-2.00334

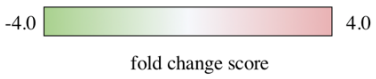


Figure 10 | PTSD associated differentially expressed genes. (A) Venn diagram of differentially expressed genes between analyses. The PTSD and high intrusion symptom associated genes were mostly upregulated (94% and 98%), whereas the PTSD improved associated genes were all downregulated. (B) Ten overlapping differentially expressed genes between all three analyses with fold change score depicting the overexpression associated with posttraumatic stress symptoms and underexpression associated with posttraumatic stress symptom reduction. Adapted from "*Gene expression differences in PTSD are uniquely related to the intrusion symptom cluster: a transcriptome-wide analysis in military service members*", by Rusch, H. L., et al., 2019, Brain, Behavior, and Immunity, 80, p. 906. © 2019 by Elsevier Inc. Adapted with Permission.

The differentially expressed gene lists from the three analyses (see Figure 10) were then uploaded into Ingenuity Pathway Analysis to calculate the top physiological systems and networks linked with each gene expression comparison. Briefly, immune response and immune cell trafficking systems were upregulated in the PTSD group and high intrusion symptom group at baseline, which were downregulated with posttraumatic stress symptom improvement at follow-up. Downregulated networks of lipid metabolism, nucleic acid metabolism, and small molecule biochemistry, with an NF-κB hub, were linked to PTSD symptom improvement following the CBT-I and APAP therapy (Figure 11).

[b] All participants with PTSD were included in this analysis, not only the participants with both baseline and follow-up gene expression data.

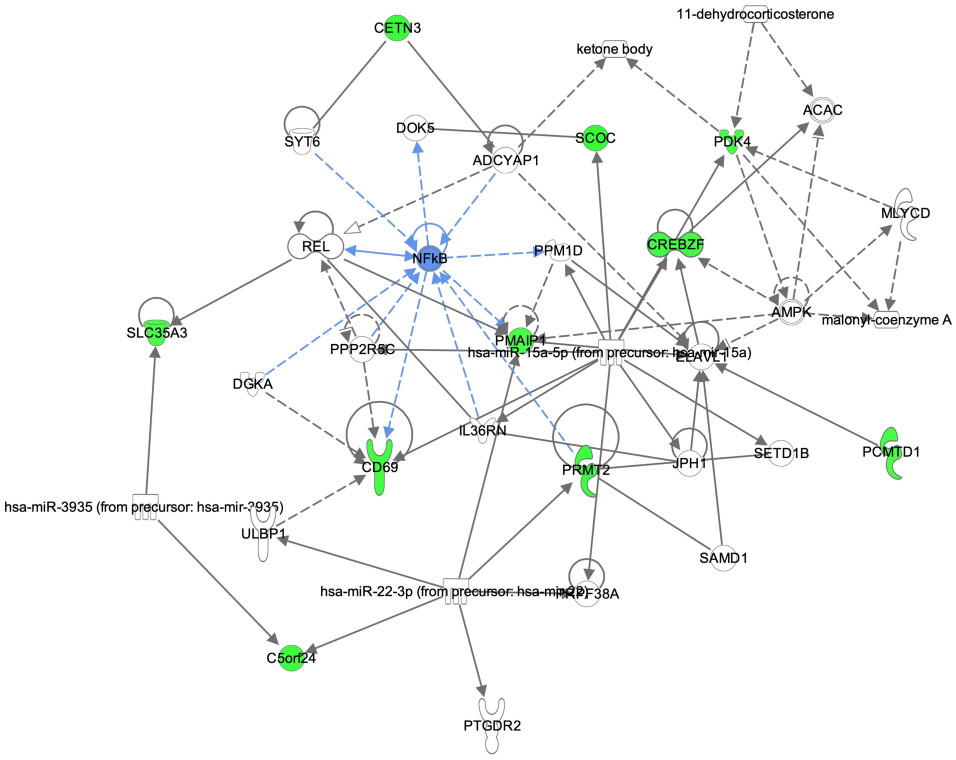


Figure 11 | Ingenuity pathway analysis. Ten genes (green) related to PTSD symptom improvement were linked to downregulated networks of lipid metabolism, nucleic acid metabolism, and small molecule biochemistry, with a nuclear factor kappa-beta (NF-κB) hub (blue). Reprinted from "*Gene expression differences in PTSD are uniquely related to the intrusion symptom cluster: a transcriptome-wide analysis in military service members*", by Rusch, H. L., et al., 2019, Brain, Behavior, and Immunity, 80, p. 906. © 2019 by Elsevier Inc. Reprinted with Permission.

4.2 Study II: Results

Study II was a meta-analysis that assessed the effect of mindfulness meditation on sleep quality. Eighteen trials with 1654 participants met the inclusion criteria and were included in the meta-analysis. In-class meditation sessions ranged from 1 to 2.5 hours per week for 2 to 16 weeks. Meditation practice was encouraged at home in all 18 trials; however, 12 trials specified a practice time, which ranged from 15 to 60 minutes daily. Seven trials offered a full-day silent meditation retreat, and one trial offered an in-class booster session two months following the completion of the main program.

The evidence suggests that mindfulness meditation is an effective treatment to improve sleep quality in adult populations with various mental and physical health conditions. Seven trials compared mindfulness meditation to evidence-based sleep treatments, such as CBT-I or medication for insomnia.[185-191] The results indicated that mindfulness meditation had a similar effect on sleep quality compared to the evidence-based sleep treatments at postintervention (ES -0.03 [95% CI -0.49 to 0.43]) and at a 5- to 12-month follow-up (ES -0.14 [95% CI -0.62 to 0.34]). However, the strength of this evidence was low due to the

high heterogeneity at both timepoints, and further studies are needed to confirm these results (Figure 12A). Eleven trials, compared mindfulness meditation to time/attention matched placebo controls.[192-202] The results indicated that mindfulness meditation was superior to the time/attention matched placebo controls at postintervention (ES 0.33 [95% CI 0.17 to 0.48]) and at a 5- to 12-month follow-up (ES 0.54 [95% CI 0.24 to 0.84]). The strength of this evidence was moderate, indicating that the findings are likely to be stable, but some doubt remains (Figure 12B).

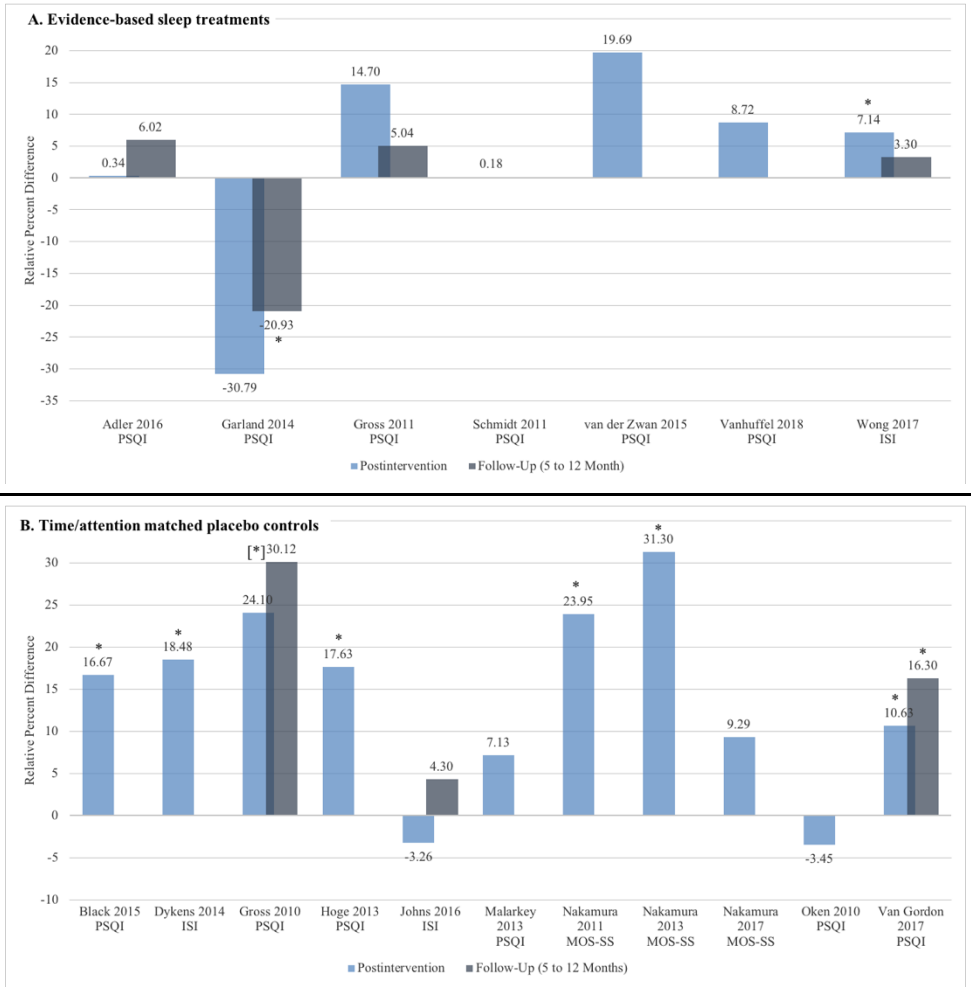


Figure 12 | Between-group relative percent difference in change score for (A) evidence-based sleep treatments and (B) time/attention matched placebo controls. Author, year, and sleep scale are noted at the bottom of each cluster bar. Follow-up scores are reported for trials with a follow-up assessment between 5 and 12 months from baseline. Percent change in sleep score was calculated using the formula: $\{[(\text{postintervention mean}^{\text{control}} - \text{baseline mean}^{\text{control}}) - (\text{postintervention mean}^{\text{meditation}} - \text{baseline mean}^{\text{meditation}})]/ (\text{baseline mean}^{\text{meditation}}) \times 100\}$. Positive scores are the relative percent change in favor of meditation. For example, a change score of 20% indicates the meditation group had a 20% higher improvement in sleep quality score compared to the control group. *The result is statistically significant per manuscript. [*]Overall group effect is statistically significant. ISI, Insomnia Severity Index; MOS-SS, Medical Outcomes Study—Sleep Scale; PSQI, Pittsburgh Sleep Quality Index. Adapted from "*The effect of mindfulness meditation on sleep quality: a systematic review and meta-analysis of randomized controlled trials*", by Rusch, H. L., et al., 2018, Annals of the New York Academy of Sciences, 1445(1), p. 11. © 2018 John Wiley & Sons, Inc. Adapted with permission.

Seventeen trials reported on in-class meditation hours for the total intervention, which ranged from 3 to 42 hours (15.6 mean, 9.8 *SD*), including the full-day retreat. There was no support for a dose-response relationship between in-class meditation hours and standardized sleep quality change scores. Six trials evaluated a dose-response relationship between at-home mediation practice and improvements in sleep quality from baseline to postintervention. Three trials found a significant positive correlation.[185,189,202] While another three trials identified no relationship.[187,191,194]

4.3 STUDY III: RESULTS

Study III investigated if sleep quality improvements, following a 4-week mindfulness-based integrative medicine program, were associated with reductions in neurobehavioral symptoms and decreases in levels of inflammatory biomarkers in sleep disturbed military service members with mTBI. The demographic and clinical characteristics for the total sample are presented in Table 6.

Table 6 | Demographic and clinical characteristics of the total sample.

	Baseline (week 1)	Follow-Up (week 4)
Variables, No. (%)		
Male sex	93 (100)	
White race	89 (95.7)	
Tobacco users	34 (36.6)	
Alcohol users	79 (84.9)	
PTSD Dx.	44 (47.3)	21 (22.6)
Anxiety Dx.	60 (64.5)	22 (23.7)
Depression Dx.	60 (64.5)	20 (21.5)
Postconcussion syndrome Dx.	81 (87.1)	31 (33.3)
Variables, mean (SD)		
Age, years	40.77 (5.35)	
Body mass index	28.56 (3.18)	
Total treatment, hours		29.24 (8.71)
Creative arts therapy		9.77 (3.77)
Mindfulness practices		6.93 (3.75)
Other integrative practices		12.55 (7.03)

All diagnoses (Dx.) are provisional based on self-report measures.

From baseline to follow-up the sample as a whole had significant reductions in sleep disturbance ($t_{73} = 6.449$; $p < 0.001$), posttraumatic stress ($t_{76} = 7.598$; $p < 0.001$), anxiety ($t_{76} = 11.077$; $p < 0.001$), depression ($t_{75} = 9.731$; $p < 0.001$), and postconcussion symptoms ($t_{76} = 10.684$; $p < 0.001$), as well as decreases in levels of TNF-α ($t_{82} = 3.246$; $p < 0.002$). No significant change in levels of IL-6 or IL-10 were observed. The next analysis sought to

determine if there was an association between sleep quality improvements and change in neurobehavioral symptom severity and inflammatory biomarker levels. Herein the data indicated that improvements in sleep quality were significantly associated with reductions in posttraumatic stress (β = 1.286; [95% CI 0.689 to 1.883]; $p < 0.001$), anxiety (β = 0.568; [95% CI 0.322 to 0.814]; $p < 0.001$), depression (β = 0.761; [95% CI 0.504 to 1.017]; $p < 0.001$), and postconcussion symptoms (β = 1.786; [95% CI 1.054 to 2.518]; $p < 0.001$) (Figure 13). Improvements in sleep quality were not directly associated with change in any of the inflammatory biomarker levels over this same 4-week period.

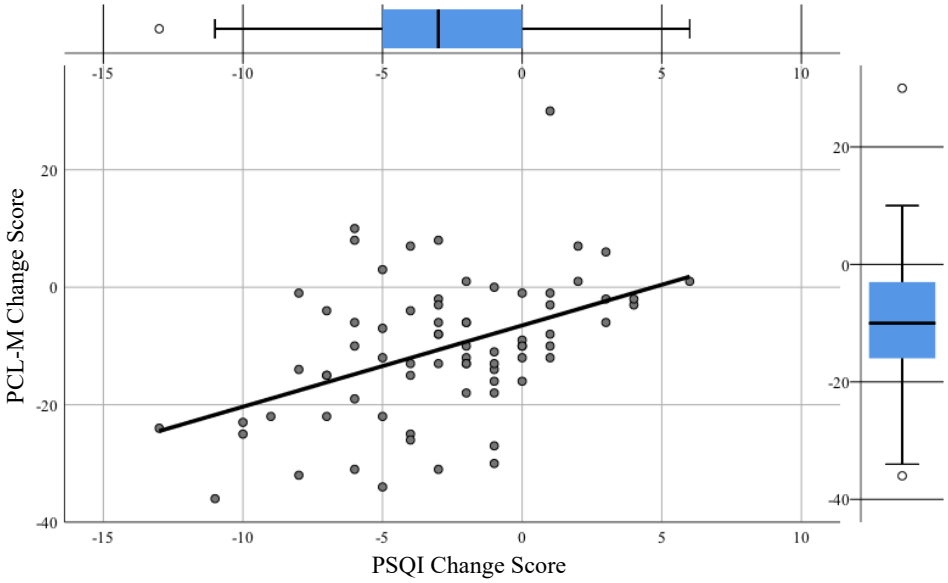


Figure 13 | Associations between sleep quality improvements and change in posttraumatic stress symptoms. Change scores were calculated by subtracting the baseline (week 1) value from the follow-up (week 4) value, hence a negative value indicates a reduction in sleep disturbance and posttraumatic stress symptoms. PCL-M, PTSD Checklist—Military Version; PSQI, Pittsburgh Sleep Quality Index

Following the 4-week integrative medicine program, 40.9% of participants reported clinically meaningful sleep quality improvements, 15.1% reported sleep quality declines, and 23.7% reported no meaningful difference in sleep quality. Of the PTSD participants, 65.8% had clinically meaningful symptom reduction (change score reduction ≤ -10). For the subgroup analysis, participants with improved sleep quality (n = 38) were compared with participants with declined sleep quality (n = 14) to examine the putative role of sleep quality changes on neurobehavioral symptom severity. Herein, there were significantly larger reductions in posttraumatic stress ($f_{1,44}$ = 13.621; p = 0.001), anxiety ($f_{1,45}$ = 5.694; p = 0.021), depression ($f_{1,44}$ = 8.605; p = 0.005), and postconcussion symptoms ($f_{1,44}$ = 6.969; p = 0.011), in the sleep improved group. (Figure 14).

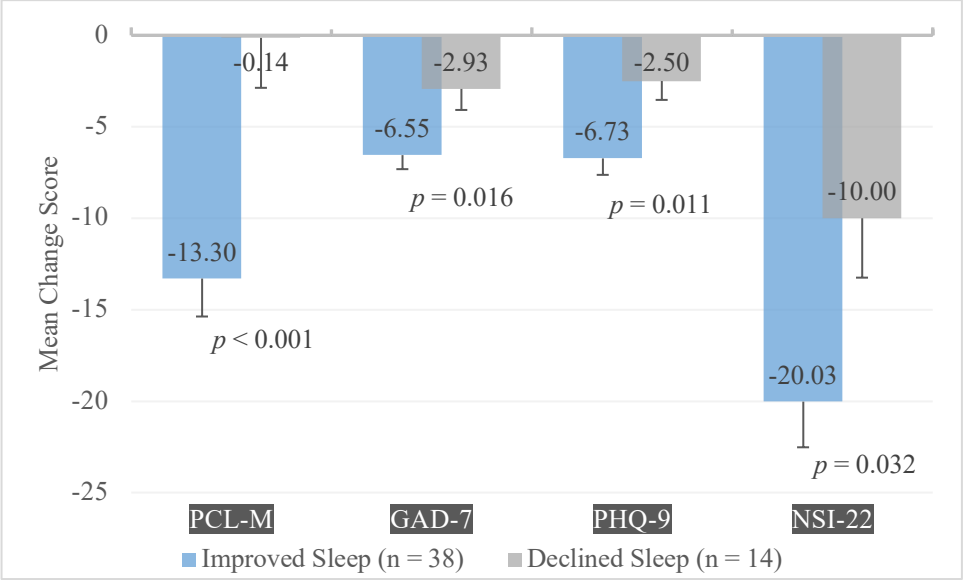


Figure 14 | Change in neurobehavioral symptom severity from baseline to follow-up according to change in sleep quality. Change scores were calculated by subtracting the baseline (week 1) value from the follow-up (week 4) value, hence a negative value indicates a reduction in neurobehavioral symptoms. GAD-7, General Anxiety Disorder 7-Item; NSI-22, Neurobehavioral Symptom Inventory 22-Item; PCL-M, PTSD Checklist—Military Version; PHQ-9, Patient Health Questionnaire 9-Item. Data are depicted as mean change score and standardized mean error.

4.4 Study IV: Results

Study IV investigated the effect of a brief 5-week (7.5-hour) mindfulness-based program on perceived stress symptoms in moderately stressed healthcare professionals. The baseline demographic and clinical characteristics for the meditation and control groups are presented in Table 7. The meditation group had a mean age in years (*SD*) of 35.7 (15.4) and the controls had a mean age in years (*SD*) of 36.4 (13.0).

Table 7 | Baseline demographic and clinical characteristics.

Variable, No. (%)	Meditation (*n* = 43)	Control (*n* = 35)
Female sex	37 (86.1)	28 (80.0)
Hispanic/Latinx	5 (11.9)	5 (14.3)
Race		
American Indian/Alaska Native	1 (2.3)	0 (0.0)
Asian	7 (16.3)	6 (17.1)
Black	3 (7.0)	2 (5.7)
White	27 (62.8)	21 (60.0)
Mixed/Other	5 (11.6)	6 (17.1)
Marital status		
Single	28 (65.1)	19 (54.3)
Married	8 (18.6)	12 (34.3)
Divorced/Separated	3 (7.0)	3 (8.6)
Widowed/Other	4 (9.3)	1 (2.9)
Medical condition	16 (37.2)	9 (25.7)
Psychiatric condition	16 (38.1)	14 (40.0)

To test the effect of the brief 5-week (7.5-hour) mindfulness-based program, the meditation group was compared with the control group on their change in symptoms and behaviors from baseline (week 1) to postintervention (week 5). Herein, the meditation group had significantly larger reductions in perceived stress (Δ -2.50 [95% CI -4.28 to -0.72] vs. Δ -0.04 [95% CI -0.37 to 0.29]; $p = 0.016$) and anxiety (Δ -2.13 [-2.79 to -1.48] vs. Δ -0.19 [-0.53 to 0.14]; $p < 0.001$], as well as significantly larger increases in positive affect (Δ 2.94 [0.70 to 5.18] vs. Δ -0.33 [-0.68 to 0.02]; $p < 0.001$], state mindfulness (Δ 1.59 [1.17 to 2.01] vs. Δ 0.26 [-0.08 to 0.60]; $p < 0.001$), and mindfulness self-care practices (Δ 1.61 [0.68 to 2.53] vs. Δ -0.16 [-0.49 to 0.17]; $p < 0.001$) (Figure 15). There were no significant between-group differences in burnout, negative affect, or trait mindfulness.

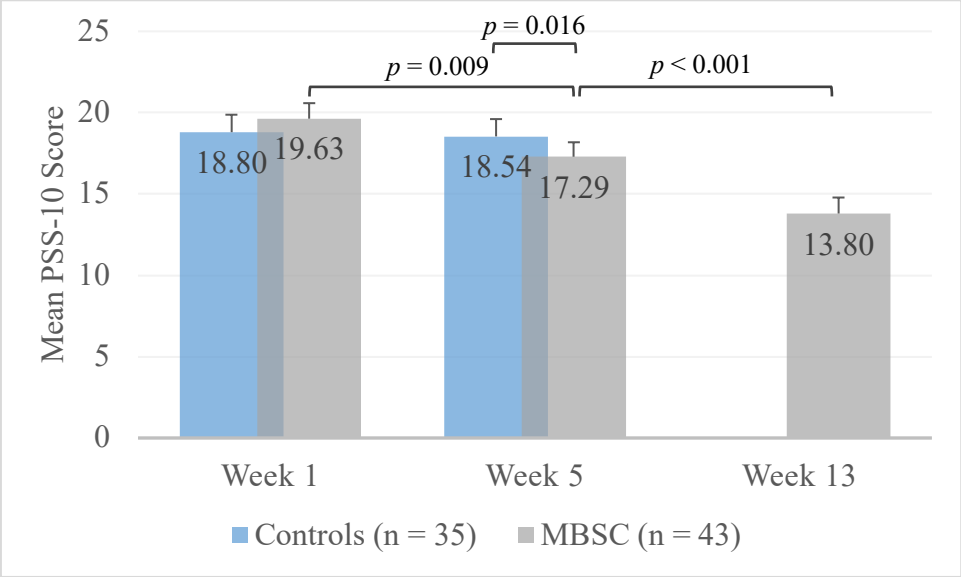


Figure 15 | Between and within-group Perceive Stress Scale-10 (PSS-10) score differences. The mindfulness-based self-care (MBSC) group had significantly larger reductions in perceived stress compared with the control group at postintervention (week 5) and these reductions were maintained through the follow-up assessment (week 13). Data are depicted as mean score and standardized mean error.

The second analysis sought to determine if the meditation group maintained any potential benefits gained from the mindfulness-based program two months later. As such, from baseline (week 1) to follow-up (week 13), the meditation group had additional significant reductions in perceived stress (Δ = -6.14 [95% CI -7.84 to -4.44]; $p < 0.001$), maintained reductions in anxiety (Δ = -1.46 [-1.97 to -0.94]; $p < 0.001$), and had additional significant increases in state mindfulness (Δ = 1.89 [1.39 to 2.39]; $p < 0.001$) (Figure 15). The increases in positive affect and mindfulness self-care practices observed from baseline to postintervention were not maintained at follow-up.

When you change the way you look at things,
The things you look at change.

— Max Planck

5 DISCUSSION

The general aim of this thesis was to examine the inextricable link between stress, sleep, and inflammation, and how gaining a better understanding of these interconnected systems could be harnessed to develop enhanced treatments for populations with stress-related symptoms and disorders. The main findings, methodological considerations, clinical implications, and directions for future research are discussed.

5.1 STUDY I: DISCUSSION

Study I investigated the effect of 4 to 8 weeks of CBT-I and APAP therapy on posttraumatic stress symptoms, as well as the gene expression pathways that may mediate this effect in sleep disturbed military service members with and without PTSD. Results indicated that 22.6% of participants with PTSD had clinically meaningful posttraumatic stress symptom reduction following treatment (i.e., response rate). The average response rate for trauma-focused psychotherapy is between 49 to 70% in military populations.[129] The lower response rate in our study seemed to be due to the high noncompliance rate to the APAP therapy. Typically, noncompliance rates of 20% or more pose serious threats to treatment efficacy—our noncompliance rate of 66.7% was three times greater than the accepted standard.[203] Even though positive airway pressure therapies, including APAP therapy, are the most effective treatment for obstructive sleep apnea, compliance is challenging in populations with PTSD, and approaches to increase adherence are needed.[204]

In the pathway analysis, genes associated with immune response systems were the most reliably upregulated pathways that distinguished participants with PTSD from those without PTSD. These results are supported by our prior transcriptome work (Paper 3) and the results from a mega-analysis of blood transcriptome studies (N = 511).[119,205] Moreover, the current study extends these findings by showing that posttraumatic stress symptom reduction, following standardized sleep therapy, is associated with a downregulation of these same immune response systems. Despite this relationship, the direction of causality cannot be determined. It is also quite possible that sleep quality improvements reduced both the severity of posttraumatic stress symptoms and levels of inflammation simultaneously and independently. Within the same cohort (but not grouping by PTSD) we previously reported that participants with improved sleep quality, following standardized sleep therapy, had reduced expression of genes related to inflammatory cytokines with a trend reduction in posttraumatic stress symptoms (Δ = -3.1 [*SD* 1.2]; $p < 0.067$) (Paper 2).[124]

In Study I, we also found that gene expression differences between participants with and without PTSD were almost exclusively attributed to the intrusion symptom cluster (98%), and there were no gene expression differences associated with the remaining two symptom clusters. One justification for these findings is that intrusion symptoms (e.g., nightmares, flashbacks, and reexperiencing symptoms) are specific to PTSD, whereas pathological symptoms of avoidance/numbing and arousal overlap with other mental and physical health conditions common in military populations, most notably depression and mTBI (Figure 16). If participants endorse PTSD-like symptoms that are due to co-occurring conditions—for example, cognitive deficits incurred from mTBI—the same phenotypic expression might be represented by different underlying gene expressions and therefore mask between-group differences. An alternative explanation could be that trauma exposure elicits gene expression alterations involved in intrusion symptoms, and secondary symptoms of avoidance, depression, hyperarousal, etc. develop in response. For example, flashbacks could cause an individual to avoid certain people and places in an effort to minimize trauma-related triggers and increase a sense of safety. It is also likely that the PTSD group in our study was comprised of false-positive cases—participants who met the PCL-M ≥ 50 cutoff score by endorsing PTSD-like symptoms due to comorbid conditions. When these potentially false-positive cases were removed from the analysis, a more robust gene expression profile emerged.

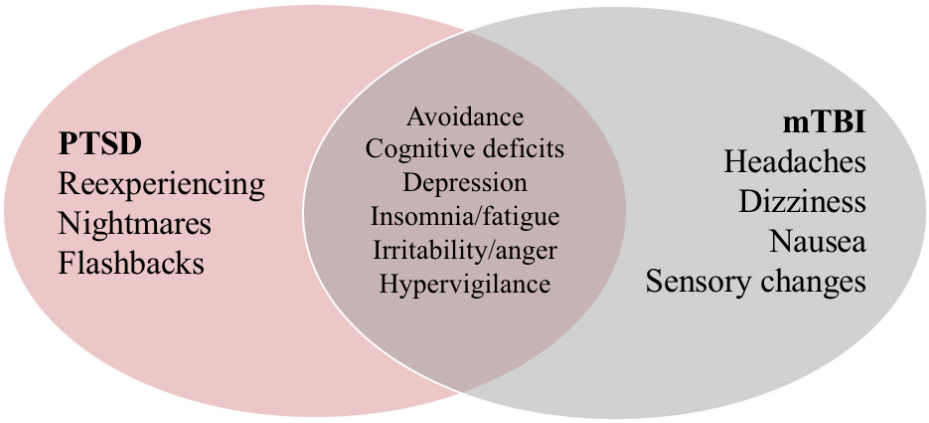


Figure 16 | Venn diagram of symptom overlap between posttraumatic stress disorder (PTSD) and mild traumatic brain injury (mTBI). Intrusion symptoms (e.g., reexperiencing, nightmares, and flashbacks) are specific to PTSD. Whereas avoidance/numbing symptoms (e.g., avoidance of people, places, and things that are trauma reminders and cognitive and mood alterations) and arousal symptoms (e.g., insomnia, irritability, and increased startle) overlap with other neurobehavioral conditions. Reprinted from "*Gene expression differences in PTSD are uniquely related to the intrusion symptom cluster: a transcriptome-wide analysis in military service members*", by Rusch, H. L., et al., 2019, Brain, Behavior, and Immunity, 80, p. 906. © 2019 by Elsevier Inc. Reprinted with Permission.

5.2 STUDY II: DISCUSSION

In order to investigate alternative interventions that may improve sleep quality and potentially provide additional benefits for stress-related disorders, Study II conducted a meta-analysis to determine the effect of mindfulness meditation on sleep quality outcomes in sleep disturbed adults with various mental and physical health conditions. The results indicated that mindfulness meditation had a similar effect on sleep quality compared to the evidence-based sleep treatments and was superior to the time/attention matched placebo controls. The results also suggested that the benefits of mindfulness meditation were maintained for up to 5 to 12 months following the completion of the study. Mindfulness research is still in its infancy; however, these long-term effects may have been supported by sleep architecture changes, functional and structural brain alterations, or increased mastery of techniques that minimize sleep-disruptive emotional and cognitive processes.[206-209] Nevertheless, due to the high heterogeneity and modest number of studies that met our inclusion criteria, we had low to moderate confidence in these results, and further research is warranted to confirm our positive findings.

We also found no evidence to support a dose-response relationship between in-class meditation hours and change in sleep quality. These results are echoed in a meta-analysis that evaluated the relationship between in-class meditation hours and change in psychological distress.[210] Dose-response relationships are arguably one of the most difficult metrics in meditation research due to a number of factors. First, it is challenging to accurately assess how mindful (verse mind wandering) a participant is during meditation practice.[211] Moreover, mediation progress has a multiphasic trajectory, which is often misunderstood in Western contexts.[212] Traditionally, success in meditation is defined by increased awareness (*sati*) and equanimity (*upekkhā*), whereby positive mental and physical states are a byproduct. When symptom change over a brief period is the only benchmark of success, meditation progress and its potential effect on wellbeing may be veiled.

5.3 STUDY III: DISCUSSION

Once mindfulness meditation was established as a potential intervention to improve sleep quality, Study III investigated if sleep quality improvements, following a 4-week mindfulness-based integrative medicine program, were associated with reductions in posttraumatic stress, anxiety, depression, and postconcussion symptoms, as well as decreases in inflammation in sleep disturbed military service members with mTBI. Results indicated that 40.9% of participants reported clinically meaningful sleep quality

improvements, following the intervention, which were linked to reductions in posttraumatic stress and other neurobehavioral symptoms. These findings were also reflected in the subgroup analysis where we compared participants with improved sleep quality to participants with declined sleep quality on the same outcomes. At follow-up, 65.8% of participants with PTSD and 55% of the entire cohort had clinically meaningful reductions in posttraumatic stress symptoms. However, the response rates were quite diverse between the improved sleep group (60%) and the declined sleep group (14%). These findings suggest that improved sleep quality is involved in posttraumatic stress symptom reduction and extends this line of inquiry to include other neurobehavioral symptoms. Nonetheless, the study design tempers any conclusions regarding the direction of causality, and symptom reduction overall may be due to placebo effects or other factors.

Next, we found a small, but significant decrease in levels of TNF-α over time; however, these reductions were not associated with improvements in sleep quality. Even though sleep plays an essential role in regulating the innate immune system, evidence of this direct relationship may not have been captured by our choice of sleep quality assessment (the PSQI self-report measure) or duration of study assessment (4-week period). In this respect, a comprehensive meta-analysis ($N > 50,000$), found that levels of inflammatory biomarkers such as CRP, IL-6, and TNF-α were subject to the diverse parameters used in clinical trials to assess sleep quality (e.g., objectively measured, clinician assessed, or self-reported), as well as the duration of sleep deprivation (e.g., years, days, one night, or part of a night).[121] It is also possible that the decreased levels of TNF-α may have been a downstream effect in response to a reduction in neurobehavioral symptoms and not in response to sleep quality improvements.[118,213] Lastly, we cannot rule out that such observation was not due to diurnal variation or natural variation over time.[214,215] We also did not observe significant changes in levels of IL-6 and IL-10 over time or in association with improvements in sleep quality. Sex differences are one possible reason for these findings.[216] The current cohort was comprised solely of men and prior studies investigating human adult molecular and protein biomarkers have more often indicated stronger links between sleep disturbance and inflammation in women than in men.[217,218] This sexual dimorphism is thought to be driven at least in part by the differences in reproductive hormones between the two sexes.[218,219]

5.4 STUDY IV: DISCUSSION

We found some evidence that posttraumatic stress symptoms were reduced in military service members following the mindfulness-based integrative medicine program; however,

this program required almost 30 treatment hours. This may be an excessive treatment duration when mindfulness meditation is used in populations with less severe symptoms. As such, Study IV investigated the effect of a brief 5-week (7.5-hour) mindfulness-based program on perceived stress symptoms in moderately stressed healthcare professionals. Results indicated that the meditation group had larger reductions in perceived stress and anxiety compared with the control group, and these reductions were maintained two months following the completion of the program. At present there are no recognized minimal clinically important difference scores for the PSS-10 (perceived stress) or the VAS-A (anxiety).[c] However, normative data from 2009 reported mean PSS-10 scores of 19.11 in high-stress groups and 11.09 in low-stress groups.[220] In our meditation group, PSS-10 scores reduced from 19.63 to 13.30 over the course of the study. Thus, we can extrapolate that the participants had some clinically meaningful level of stress reduction, although their levels may not have reached the threshold of low stress. However, it's worth noting that the participants were self-motivating volunteers and may have been prone to an expectancy bias, which could have influenced the between-group effects.[d] We also found no significant between-group differences in burnout (i.e., emotional exhaustion and depersonalization), which may have been due to a floor effect since the participants did not present with high levels of burnout to begin with. Moreover, the use of the abridged 2-item MBI may not have captured the presentation of burnout in this population.

Typical mindfulness-based programs are around 30 hours in duration and include a full-day silent meditation retreat. This can be cost prohibitive and impractical to implement during the workday in a bustling healthcare setting.[221] When mindfulness-based programs are below four hours, the benefits are inconclusive.[221] In the current mindfulness-based program, classes filled up within three days of the announcement, which was a proxy for community interest. The overall quality of the program was rated as 'very good' or 'excellent' by 97% of the meditation participants. No adverse events were reported and the attrition rate of 19% was consistent with the literature for comparable populations.[222] As such, these findings suggest that a brief 5-week (7.5-hour) mindfulness-based program is feasible and effective at reducing perceived stress and anxiety in healthcare professionals.

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[c] The minimal clinically important difference (MCID) defines the smallest amount an outcome must change to be meaningful to the patient. An outcome's change can be statistically significant and not meet the MICD.

[d] Expectancy bias is a type of cognitive bias that occurs when a participant expects a certain result and therefore unconsciously manipulates an experiment or reports the expected result.

5.5 General Methodological Considerations

This thesis had a number of strengths and limitations; the main methodological considerations are discussed below, and a comprehensive account can be found in the corresponding manuscripts (see Chapter 9. Appendix). The first methodological consideration involves the inclusion/exclusion of randomized controlled trials. Randomized controlled trials are the gold standard for evaluating the effectiveness of an intervention. Herein, groups of similar people are randomly assigned to an experimental group or to a control group; the outcome variable of interest is the only expected difference between the two groups. However, due to ethical, feasibility, and financial reasons this study design is sometimes not a viable option. In Study II, we only included randomized controlled trials in the meta-analysis that compared mindfulness meditation to evidence-based sleep treatments or to time/attention matched placebo controls. This provided greater confidence that the reported benefits of mindfulness meditation were not due to nonspecific effects.[e] In Study IV, we randomized participants to either a meditation experimental group or a life-as-usual control group. Including an active control group would have strengthened our study; however, there is no consensus on what constitutes an ideal active control group for mindfulness meditation in non-clinical populations. Sham mindfulness meditation has been used with success for very short interventions (20 to 30 minutes).[223] However, administering sham mindfulness meditation for longer programs over multiple sessions becomes challenging. In 2012, researchers attempted to validate the Health Enhancement Program (HEP) as an acceptable active control group for mindfulness mediation; herein all components of MBSR are matched in the HEP control group with the exception of mindfulness.[224] While these types of studies are critical in evaluating the effectiveness of mindfulness meditation, they can be costly and timely to implement. For ethical reasons, Study I and Study III were not randomized controlled trials (it is unethical to deny treatment to symptomatic active duty military), thus we were limited in the conclusions we could make regarding the treatment effects for these studies.

One way we attempted to compensate for the single-arm designs in Study I and Study III was to minimize clinical heterogeneity. Clinical heterogeneity arises from differences in participant demographics (e.g., sex, age, race), clinical characteristics (e.g., symptom

[e] Nonspecific effects are the indirect benefits of the treatment, such as placebo effects and indirect benefits from the healthcare providers' time and attention toward the participants.

severity, comorbidities), and intervention characteristics (e.g., type, duration, frequency, dose). This variability can lead to inaccurate conclusions and in turn mislead decision-makers and other researchers. The participants in Study I and Study III were mostly white men under 57 years of age. Both studies were also comprised of military service members with sleep disturbance and neurobehavioral symptoms. However, in Study I the most conservative cut-points were used to create well-characterized groups of participants with and without PTSD. This allowed us to determine the differentially expressed genes that were specific to posttraumatic stress symptoms and symptom reduction with enhanced precision. In Study III, there was much greater heterogeneity in symptom severity and comorbidity. While most participants had some degree of neurobehavioral symptoms, only 47 to 87% met criteria for a provisional diagnosis for at least one mental health condition (range dependent on the condition, see Table 6). This may have masked changes in IL-6 and IL-10 over time that could have been linked to the improvement of a specific symptom or condition. Lastly, there was unintended heterogeneity in the intervention characteristics. In Study I, the participants were assigned a standardized sleep treatment based on their diagnosed sleep disorder; however, there was such a high noncompliance rate (66.7%) for the APAP therapy, it was difficult to draw any conclusions regarding treatment effects except that the APAP therapy was not feasible in participants with PTSD. In Study III, the participants, in collaboration with their practitioner, designed their own treatment plan; this personalized medicine approach is at the heart of integrative medicine but precluded us from testing for specific intervention effects.

While clinical homogeneity has its strengths, it should be balanced with generalizability. Generalizability is the extent to which the conclusions of a study can be applied to broader groups of people and settings. Even though Study II had very strict inclusion/exclusion criteria when it came to intervention types and comparison groups, we were liberal in including all adult populations provided they had clinically relevant sleep disturbance and were not expert meditators (see Table 3). Since the final meta-analysis included adults with an array of mental and physical health conditions, there is greater confidence that the findings can be extended to other groups with sleep disturbance beyond those that were included in our study. Study I and Study III are limited in their ability to generalize the findings to women, civilians, and other non-military trauma types. Likewise, in Study IV, the results may not generalize to men, lower educational levels, or other institutes that do not value or emphasize the benefits of supporting employee health and wellness.

5.6 Clinical Implications & Future Directions

PTSD can be difficult to diagnose because it shares symptoms with other mental and physical health conditions. When PTSD is misdiagnosed, patients do not receive the correct treatment, which can be inefficient and harmful. In Study I, we investigated what symptoms could genetically differentiate participants with PTSD from those without PTSD and found that intrusion symptoms accounted for almost all of the gene expression differences. Additionally, we found that intrusion symptoms were linked to increased expression of immune response genes, which were normalized with symptom reduction. This highlights the importance of focusing on the intrusion symptom cluster for precision medicine initiatives in individuals with PTSD. Since Study I was initiated, more advanced gene expression technologies like RNA sequencing are now available that are able to identify both known and unknown genes. A significant body of literature has also demonstrated that the protein and microRNA constituents of exosomes (small extracellular vesicles) hold promise as novel biomarkers for PTSD (Paper 5).[225] This unveils exciting opportunities for improving diagnostics in trauma-exposed populations. Our ultimate goal is to design biomarker-based tests that will not only predict who will develop PTSD following a traumatic event but will also identify the most effective treatment for a given individual.

Another avenue to help decrease the risk for PTSD and increase the effectiveness of treatments is through the early identification and treatment of sleep disturbance in trauma-exposed populations. At present, there is no consensus about incorporating evidence-based sleep treatments into standard of care for the prevention or management of PTSD; importantly, there are no empirical data available to establish the order in which sleep and PTSD treatments should be delivered.[226] One recent pilot study examined the delivery of CBT-I prior to trauma-focused psychotherapy in veterans and reported large decreases in insomnia and posttraumatic stress symptoms, and large increases in quality of life.[227] Moreover, clinicians need to be aware that sleep disturbance can diminish the efficacy of trauma-focused psychotherapy, especially when they rely on memory extinction and consolidation processes.[99,228] Future studies might establish the efficacy of integrative medicine approaches that address sleep disturbances and posttraumatic stress symptoms simultaneously. For example, Study III combined soporific mindfulness practices with art therapy, which has a putative effect on PTSD trauma processing.[229] Lastly, while APAP therapy may be more challenging for individuals with PTSD, research has found that enhanced self-efficacy can help increase compliance.[230]

In addition to evidence-based sleep treatments, alternative interventions that target multiple biological systems may offer additional benefits to individuals with stress-related disorders. Current first-line treatments for PTSD target psychological symptoms; however, the presence of immune response genes (Study I) suggests a need for a more comprehensive treatment approach to address the potential influences of immune dysregulation on emotion and behavior.[231,232] It is possible that the growing interest in alternative therapies for PTSD such as meditation, yoga, and other interventions that increase physical activity or alter dietary intake may provide benefits through their anti-inflammatory effects.[233-236] To date, at least 20 clinical trials have investigated the antidepressant activity of anti-inflammatory treatments on depressive symptoms in individuals with chronic inflammatory conditions.[237] Many of these trials reported significant reductions in symptoms of depression, which prompted a new series of clinical trials specifically designed to evaluate the safety and efficacy of anti-inflammatory therapeutics in mood disorders.[238] This research opens up a new line of investigation for novel therapeutics that directly target inflammation in individuals with PTSD, which could potentially result in improved mental and physical outcomes. Future research would also benefit from empirical studies that examine the relationship between posttraumatic stress symptoms and inflammation dimensionally (along a spectrum from low to high) as outlined by the Research Domain Criteria.

Lastly, mindfulness meditation may be beneficial to reduce perceived stress, which could potentially prevent the development of more severe mental and physical health conditions. In Study IV, the 7.5-hour mindfulness-based program was a feasible and effective level of training to reduce stress and anxiety in busy healthcare professionals. When stress is left unchecked it can increase the risk for burnout (see Figure 1). A systematic review of 36 prospective trials found that burnout was a significant predictor of developing the following physical health conditions: cardiovascular disease, hypercholesterolemia, type 2 diabetes, respiratory issues, gastrointestinal problems, musculoskeletal pain, headaches, prolonged fatigue, and mortality under 45 years of age.[239] Moreover, burnout increased the risk of developing insomnia, being hospitalized for a psychiatric disorder, and being prescribed psychotropic and antidepressant medications.[239] Burnout not only affects the individual, but has been associated with decreased productivity, suboptimal patient care, and increased medical errors.[240] To help ensure the safety of both provider and patient, organizations should work towards implementing feasible and effective interventions to build resilience in healthcare professionals, so they can better manage occupational challenges.

If I have seen further,
It is by standing upon the shoulders of giants.

— Sir Isaac Newton

6 CONCLUSIONS

The findings of these four studies led to some important conclusions regarding the link between stress, sleep, and inflammation that may inform the development of enhanced treatments for populations with stress-related symptoms and disorders. In Study II, while mindfulness research is still in its infancy, our preliminary results suggest that mindfulness meditation is effective in improving sleep in adult populations with various mental and physical health conditions. Less intense mindfulness meditation programs (7.5 hours) may be beneficial to reduce perceived stress, which could potentially prevent the development of more severe mental health conditions (Study IV). While 22.6% of individuals with PTSD had reduced posttraumatic stress symptoms following standardized sleep therapy (Study I), 65.8% of individuals with PTSD had reduced posttraumatic stress symptoms following the mindfulness-based integrative medicine program (Study III). There was some evidence that a relationship exists between sleep quality improvements, decreases in gene expression levels of inflammation, and reductions in posttraumatic stress symptoms; although the direction of causality could not be determined (Study I and III). Despite these findings, the potential mediating role of inflammation between sleep disturbance and posttraumatic stress symptoms is still unclear. Future research would profit from addressing the outstanding methodological design limitations, in addition to testing for sex differences, and using a combination of objective and subjective measures of sleep in concert with molecular and protein biomarker assessments. The field of mindfulness and psychoneuroimmunology present great opportunities for therapeutic discovery to increase resilience to buffer stress-elicited changes in physiology including immune responses.

A human being is a part of the whole, called by us "Universe", a part limited in time and space. He experiences himself, his thoughts, and feelings as something separated from the rest—a kind of optical delusion of his consciousness. This delusion is a kind of prison for us, restricting us to our personal desires and to affection for a few persons nearest to us. Our task must be to free ourselves from this prison by widening our circle of compassion to embrace all living creatures and the whole of nature in its beauty. Nobody is able to achieve this completely, but the striving for such achievement is in itself a part of the liberation and a foundation for inner security.

— Albert Einstein

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My brain is only a receiver,
In the Universe there is a core from which we obtain knowledge, strength, and inspiration.
I have not penetrated into the secrets of this core,
But I know that it exists.

— Nikola Tesla

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Let yourself be silently drawn by the strange pull of what you really love.
It will not lead you astray.

— Rumi

9 APPENDIX